Journal of Root Crops, 2011, Vol. 37 No. 1, pp. 77-85
Indian Society for Root Crops
ISSN 0378-2409

Physico-Chemical Analysis of *Colocasia esculenta* Starch

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Received: 16 December 2010; Accepted: 24 March 2011

Abstract

The physico-chemical properties of *Colocasia esculenta* (var. Ghee Kochu) starch was studied in detail. The starch was isolated and analyses were done for microscopy, X-ray diffraction (XRD), furier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) characterizations. The chemical characterizations were done using the investigation parameters like amylose content, both apparent and total, amylose leaching, acid and enzymatic hydrolysis and solubility and 1'-1'diphenylpicryl-hydrazyle (DPPH) scavenging in methanol, water and dimethyl sulfoxide (DMSO). The crystallinity of the starch (41.20%) was found to be high as reported for *C. esculenta* starches and the starch was A-type. The amylose content of the starch was found to be very poor as compared to the previous reports. The size of the starch granules was found to be 0.50-3.5 μm. An average of 42.27% of degradation was observed during 15 days of acid hydrolysis process. The enthalpy of gelatinization was found to be 13.1 J g^{-1}. Swelling power of the starch granules at room temperature was found to be 81.99±4.32%.

Key words: *Colocasia esculenta*, starch, antioxidant, physical property, chemical property

Introduction

Starch is considered as one of the strategic materials for the future. Its consumption is increasing as food and non-food. The highest utilization of the starch biopolymer is in paper, textile and chemical industries, where it serves as the raw material for the production of many items. Edible aroids are subsidiary food in tropical and subtropical countries (Hoover, 2001).

It is important to determine the composition of food commodities for the dietary purpose. Several authors evaluated the chemical composition of corms of the aroids, tannia (*Xanthosoma sagittifolium*) and taro (*Colocasia esculenta*) (Hoover, 2001). Cocoyam or tannia, the neglected crop, composed of 6% average protein and 390 calories energy per 100 g dry matter. Agbor-Egbe and Richard (1990) compared the composition of 32 cultivars of *X. sagittifolium* and *C. esculenta*. They reported differences among the species. The observation supports the work done by Rasper and Coursey (1967) which indicated that the composition of food commodities were dependent on variety, location, season, method of processing and storage. In North East India, the starchy crop, potato (*Solanum tuberosum*) is normally grown in the winter; taro, tannia (*Colocasia* and *Xanthosoma* spp.) and *Amorphophallus* sp. (grown widely) are cultivated in the rainy summer (March to August). Only few reports were available on the systematic assessment of *C. esculenta* (Lakhanpaul et al., 2003). *Colocasia* species originated from the North Eastern region of the country and spread to other parts of India and the world (Kuruvilla and Singh, 1981). The cormels of the var. Ghee Kochu is long, cone shaped and tapering towards the distal part. The length of the cormel varies from 12-17 cm, diameter 2-4 cm; either straight or smooth band at distal tapering end. Environment influences size and distribution of starch granules (Lindeboom et al., 2004).

The starch type depends on the environmental condition

in which the plant grows. In *Hordeum* spp., the stresses induced by high temperature tends to reduce the number of A-type starch granules, but proportionately the effect is less in the case of B-type granule (Tester et al., 1997). Langeveld et al. (2000) hypothesized that small granules are formed in vesicles, bobbed off from out-growth of A-type granules containing amyloplast. The hypothesis was supported by the presence of B-type granules as revealed through transmission electron microscopy and confocal laser scanning microscopy. Raeker et al. (1998) reported the presence of significant difference in amylose content among aroid cultivars. The negative correlation between amylose content and starch granule size explain, at least in part, cultivar dependency of granule size. Starch being a low-cost and renewable resource, could be used as fillers in the development of biodegradable polymer products (Ratto et al., 1999). Starch with smaller granules show slightly higher viscosity in the starch filled poly-hydroxyl-ester-ether composites (Zhou et al., 2000). Plastic film incorporated with starch produce porous structures enhancing accessibility of plastic molecules to oxygen and micro-organisms (Lim et al., 1999; Ahamed et al., 1996). Starch morphology and granule size are genetically controlled. It is also known that genetic variation and environmental condition influence structure and properties of starch (Debon and Tester, 2002; Tester et al., 1997). Therefore, an effort has been made to determine the granule size, crystallinity, composition and physico-chemical properties of starch present in *Colocasia esculenta* var. Ghee Kochu grown in North East India.

Materials and Methods

Colocasia esculenta was grown under field condition at Tezpur University, Tezpur District, Sonitpur, Assam.

Amylose, amylopectin, α-amylase and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were obtained from Himedia Laboratories. Methanol and DMSO (dimethyl sulfoxide) were purchased from Merck. HPLC grade methanol was used for DPPH scavenging assay.

Mature cormels were collected from uprooted plants. After uprooting, cormels were washed thoroughly to remove the dirt and skin. The cormels were then cut into square of about 1-3 cm^3, 100 g was weighed and soaked in double volume of potassium-metabisulfite at the concentration 50 mg l^{-1}. Starch isolation was done following the method of Jayakody et al. (2007) with minor modifications. The blended cormel paste was suspended in a double volume of extraction solution. The suspension was filtered through a double layered cheese cloth. The process was repeated twice. The filtrate was centrifuged at 1000 x g for 30 min at room temperature. The process was repeated four times with double distilled water. The upper brown layer contaminating proteins and polysaccharides was removed. The lower whitish part containing starch was isolated and washed with distilled water thrice to obtain pigment free white starch.

Proximate composition like moisture, ash, nitrogen, lipids was performed using AACC method (1990). The free lipids were extracted using chloroform: methanol (2:1, v/v) at 25°C. The bound lipid was estimated using the chloroform/methanol treated lipid extraction using *n*-propanol: water (3:1, v/v) for 7h at 50°C. The total lipid was estimated by hydrolyzing starch in 24% HCl and then extracting thrice with equal volume chloroform: methanol (2:1, v/v) at 25°C. Total phosphorus was estimated by the method described by Morrison (1964).

To determine the starch granule size, scanning electron microscopy (SEM) of the starch was done using JEOL model no. JSM-6390LV (Oxford Instrumentation Ltd.). For the analysis, the dried starch powder was sprinkled on the carbon tape and then coated with 30 nm platinum coat using JOEL auto fine coater (model no. JFC-1600). The SEM was operated at 15KV under 1 Pascal pressure with the spot size fixed at 40.

Apparent and total amylose content was determined using the method described by Gunaratne and Hoover (2002).

Starch (20 mg, dry basis) was dissolved in 90% dimethylsulfoxide (8 ml) [DMSO] in screw cap reaction vials. The contents were vigorously mixed for 30 min and then heated in a water bath (shaking intermediately) at 85°C for 20 min. The vials were then cooled to ambient temperature and the contents were diluted with water to 25 ml in a volumetric flask. One ml of diluted solution was mixed with water (40 ml) and 5 ml of I_2/KI solution (0.0025 M KI) and then adjusted to a final volume of 50 ml. After allowing the content to stand at ambient temperature the absorption was measured at 600 nm.

The total amylose content of the sample was determined

after defating with hot n-propanol: water (3:1, v/v) for 7 h. In order to correct over estimation of apparent and total amylose content, amylose content was calculated from a standard graph prepared using potato amylose and amylopectin over the range of 0-100% amylose and 100-0% amylopectin.

The method followed for estimating starch damage was based on the principle that amylose leached from the mechanically damaged starch granule was more rapidly extracted by sodium sulphate when compared to native starch granules.

The starch granule damage was estimated following the method of Medcalf and Gilles (1965). Starch (1 g) was extracted with 25 ml of extraction solution (sulphosalicylic acid 2 g l^{-1} 1.41 M sodium sulphate) for 15 min at 50°C with thorough shaking at intervals of 5 min. Celite (0.25 g) was added to the suspension followed by brief stirring. The mixture was allowed to stand for 1-2 min and later filtered through Whattman no. 1 filter paper. Ten ml aliquot from the above suspension was mixed with 10 ml of diluting solution (gelatin 50 g and 2.5 ml of H_2O_2 in 500 ml boiled distilled water) and 0.5 ml of iodine reagent (0.55 g I_2 and 1.10 g KI in 25 ml distilled water) was added. This mixture was kept in a water bath at 30°C for 15 min. The absorbance was measured at 560 nm against reagent blank. Fifty units of iodine absorbance value corresponding to 9% starch damage were used as standard for calculations.

The *C. esculenta* starch was analyzed by powder X-ray diffraction method for quantitative phase identification following the method of Gunaratne and Hoover (2002). The X-ray powder diffraction patterns were obtained using Miniflex goniometer (Rigaku Corporation, Japan) with scanning mode 2θ; scanning type being continuous; X-Ray 30 KV/ 15mA; divergence slit being variable; scattering slit 4.2°, receiving slit 0.3 mm; step 0.02 and using Kb filter from 10-40°. The starch was equilibrated at 100% relative humidity for 24 h at 25°C prior to analysis. The equation of the degree of crystallinity is as follows:

$$X_C\left[\frac{A_C}{A_C - A_A}\right]\text{x}100$$

Where,

X_C refers to the degree of crystallinity in percentage; A_C refers to the crystallized area on the X-ray diffractogram; A_A refers to the amorphous area on the X-ray diffractogram.

The Furier Transform Infrared Spectroscopy (FT-IR spectrum) of *Colocasia esculenta* starch was recorded on Nicolet IR spectrometer at room temperature. The starch powder was blended with KBr powder and pressed into tablets before measurement. A region from 400 to 4000 cm^{-1} was used for scanning.

Amylose leaching was assessed by heating the isolated starch (2mg ml^{-1} deionised water of volume 10 ml) at 60, 70, 80 and 90°C for half an hour (Gunaratne and Hoover, 2002). The tubes were centrifuged at 2000 x g for 10 min. The supernatant (4 ml) was taken out to determine amylose content following the method of Gunaratne and Hoover (2002).

Starch was hydrolyzed in triplicate with 2.2 M HCl (1 g starch/40 ml, 2.2 M HCl) at 35°C in a water bath (Pharmacia) for a period of 15 days. The starch slurry was vortexed daily to re-suspend the deposited starch granules. Aliquots taken for analysis during the interval were neutralized with 2.2 M NaOH and centrifuged (2000 rpm, 10 min). The amount of total reducing sugar (glucose equivalent) in the supernatant was determined by Somogyi-Nelson method. The extent of hydrolysis was calculated using the following formula given by Jayakody et al. (2007):

$$\text{Hydrolysis (\%)} = \frac{\text{Reducing sugar (as glucose) x 0.95 x100}}{\text{Initial starch wei ght (g)}}$$

Enzymatic digestibility of starch was assessed following the method of Jayakody et al. (2007). In short, an amorphous suspension of α-amylase in 2.9 M sodium chloride containing 3 m M calcium chloride (Himedia, India), in which the concentration of α-amylase was 32 mg protein ml^{-1} and specific activity 1,122 U mg^{-1} proteins. Starch granules (20 mg dry weight) were suspended in 10 ml 0.02 M phosphate buffer (pH 6.9) containing 0.006 M NaCl. α-amylase (5.5 μl) was added, the mixture gently mixed and digested at 37°C in a water bath (New Brunswick Scientific) for 72 h. The reaction mixture was vortexed regularly at an interval of 12 h to suspend the deposited granules. The digestion reaction was terminated by adding 5 ml of absolute ethanol. The hydrolysate was recovered by centrifugation at 2000 rpm for 5 min. Aliquots

of the supernatant were analyzed for reducing sugar (maltose) content following the method of Somogyi-Nelson

$$\text{Hydrolysis}\,\% = \frac{\text{Reducing sugar(maltose) x 0.95 x 100}}{\text{Initial starch weight (g)}}$$

Gelatinization parameter was measured using DSC equipped with thermal analysis data recording software. Water (11 μl) was added to starch with a micro syringe (3mg) in the DSC pan, which was then sealed, reweighed and allowed to stand for 2 h at room temperature in order to attain an even distribution of water. The scanning temperature range and heating rates were 20 – 120°C/min. An empty aluminium pan thermogram was taken as reference in all measurements.The transition temperature reported is the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and baseline under the peak and expressed in joul per unit weight of dry starch (J g^{-1}).

Light transmittance of *C.esculenta* starch solution (1%) in DMSO, water and methanol was measured. The absorption of the solution was recorded for 4 hours starting from t=0 with an interval of 30 min for DMSO as solvent and for water and methanol it was measured on daily basis up to four days at 650 nm on a Hitachi U-2001 at room temperature against corresponding solvent blank. The corresponding DPPH scavenging assay was done following the method described by Serpen et al. (2007) for insoluble material antioxidant capacity assay with minor modifications. In short, various concentrations of suspensions were applied to 1 ml 100 μ M of DPPH solutions along with their corresponding solvent blank. The first day reading was measured after 210 min of suspending the granules in all the solvents. The absorption of the reaction mixture was measured after 30 min at 517nm against a solvent blank. The percentage of DPPH scavenging was measured using the formula:

$$\text{DPPH scavenging}\,\% = \left[\left(\frac{A_B - A_S}{A_B}\right) \text{x} 100\right]$$

Where, A_B was absorption of DPPH treated with same volume of solvent

A_S was absorption of the DPPH treated with samples in suspension

The procedure described by Sugimoto et al. (1986) was used with slight modification. Starch (5g) was added to 75 ml distilled water in 100 ml centrifuge bottle. The bottle was stoppered and agitated on a magnetic stirrer for 1 hour, then centrifuged for 10 min at 2200 g. The water was decanted and the bottle was allowed to further drain for 10 min and weighed. The amount of water held by starch was determined. The binding capacity was calculated from the formulae:

$$\text{Water binding capacity}\,\% = \left[\frac{\text{(Final weight of the starch - Initial weight of the starch) (g)}}{\text{Initial weight of the starch (g)}}\right] \text{x} 100$$

Results were obtained in triplicates from the three different experiments. The average and standard deviation were calculated using Microsoft Excel, Version 2003.

Results and Discussion

The moisture content of the starch granules was found to be 11.23±0.2 (Table 1). The moisture content of the granule was a very important feature because the internal moisture content correlates with some physical properties of the granules like gelatinization temperature. Several reports indicate that environmental conditions affect sharply the starch and biochemical and physical properties of corm. In the present investigation also corms were found to contain higher (80%) moisture compared to normal (65-70%) moisture range of *C. esculenta* corms, but their starch granules maintained the type specificity (A type) and granule moisture level (Table 1, Fig. 1).

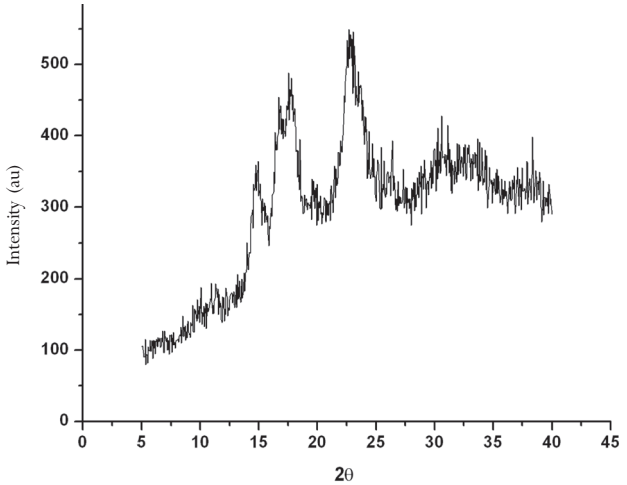

Fig. 1. X-ray diffraction pattern of the *C. esculenta* starch granules

Table 1. Proximate composition of the starch granules of *Colocasia esculenta*

Moisture (%)	Ash (%)	Nitrogen (%)	Lipid (%)			Amylose content (%)		Phosphorus (%)	Granule shape
			Solvent extracted		Acid hydrolyzed	Apparent	Total		
			Chloroform-ethanol	n-propanol – water					
11.23±0.2	0.23±0.01[a]	0.06±0.01[a]	0.03±0.01[a]	0.01±0.00[a]	0.04±0.01[a]	13.45±0.71	15.85±0.63	0.02±0.01[a]	round polygonal

[a] The data represent mean from generated data set

Ash and nitrogen content were 0.23% and 0.06 % of the dry weight. Starch bound phosphorus was found to be 0.02%, which suggests that other minerals were also present in the starch. The mineral content of the starch depends a lot on the soil mineral composition throughout its deposition seasons. Differences were observed between the surface bound and total lipid content of the granules. The amylose content after removal of lipids using n-propanol: water (3:1, v/v) was 15.85% (Table 1) suggesting that the percentage of total amylose complexed with starch amylose was 15.14%.

Scanning electron microscopic examination

As shown in Fig.2 the morphology of the starch was found to be peculiar

Fig. 2. Scanning electron microscopic image (5,500X) of *C. esculenta* starch granules showing granule size and morphology.

irregular shape. The size ranges from 0.50-3.5 μm. As per earlier reports *C. esculenta* starch granules are small rounded, ellipsoidal-truncated and poly helical shaped granules (Hoover, 2001). The granule number distribution was found to increase from 0.5 μm to 2.0 in a continuous pattern which sharply decreased after 2.0 μm and found to be smallest between 3.0-3.5 μm.

The range of starch granules from plant sources varies from 1-100 μm (Hoover, 2001). The *Colocasia* species normally have round shaped starch granules with a range of 1-5 μm. The observed value was well within the ranges.

X-ray diffraction analysis

The X-ray diffraction pattern of starch is shown in Fig. 1. The starch extracted from *Colocasia esculenta* was found to be A-type starch with characteristic peaks at $2\theta = 15.3$, 17.0, 18.0, 20.0 and 23.4°. They also differ in the content of intra-helical water. The crystalline region was found to be (X_C) 41.20 % as calculated from Fig. 1. A-type starch (mostly

cereals) exhibits reflection at 15.3, 17.0, 18.0, 20.0 and 23.4° 2θ angles. They also differ (B>A) in the content of intra-helical water. The double helices of A and B-type starch are packed in a pseudo hexagonal array. The lattice associated with B type starch has a large void (channel) which can accommodate 36 water molecules. However, in A-type starch, the lattice contains a helix in the center rather than a column of water. In both A and B-type starch, there is a spacing of double helix that corresponds to 1.1 nm distance between the axes of the two double helices (Imberty, 1988).

FTIR of *C. esculenta* var. Ghee kochu starch

The FT-IR pattern of the starch granules is presented in Fig.3. A wide band observed at 3331.91 cm^{-1} can be attributed to the O-H stretching of the starch and its width was ascribed to the formation of inter and intramolecular hydrogen bonds. The characteristic peak of starch between 1019 and 1156 cm^{-1} could be attributed to C–O bond stretching. The peak observed between 1019 and 1021 cm^{-1} could be ascribed to C-O stretch in C-O-C of starch, and the peaks near 1081 and 1154 cm^{-1} may be due to C–O stretch in C–O–H of starch. The peak near 2930 cm^{-1} might be attributed to the asymmetric stretching of C–H, while the band near 1644 cm^{-1} was ascribed to adsorbed water and the bands near 1420 and near 1368 cm^{-1} to the angular deformation of C–H.

Amylose leaching

Amylose leaching pattern of the starch at different temperature is depicted in Fig. 4. As evident from the figure there was very less leaching at 60°C where as with increasing temperature (70 and 80°C) there was an increasing rate of leaching, which got a mild break after 80°C. The decrease in the rate of leaching after 80°C might be attributed to the tightly bound amylose molecule.

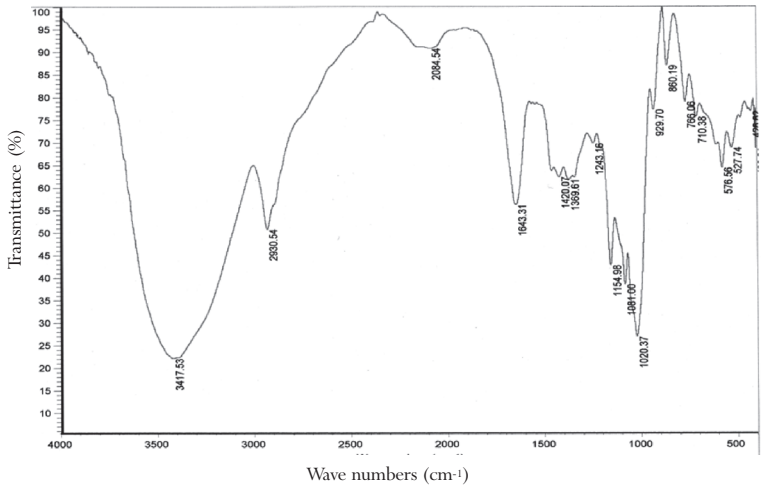

Fig. 3. FT-IR spectroscopy of the starch granules

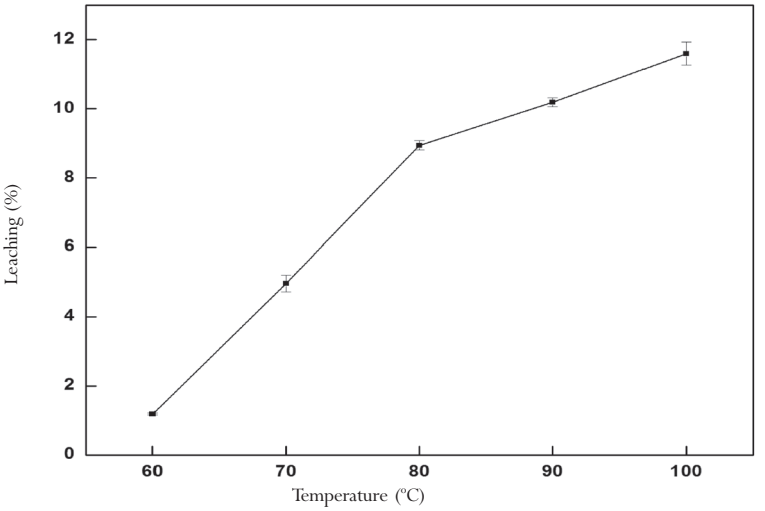

Fig. 4. Amylose leaching from the starch granules with respect to the temperature treatment

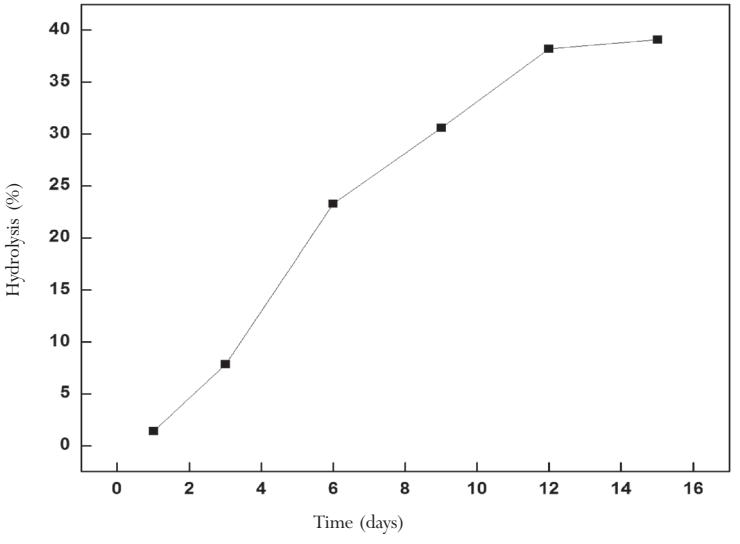

Fig. 5. Hydrolysis of the starch granules with respect to time due to acid treatment

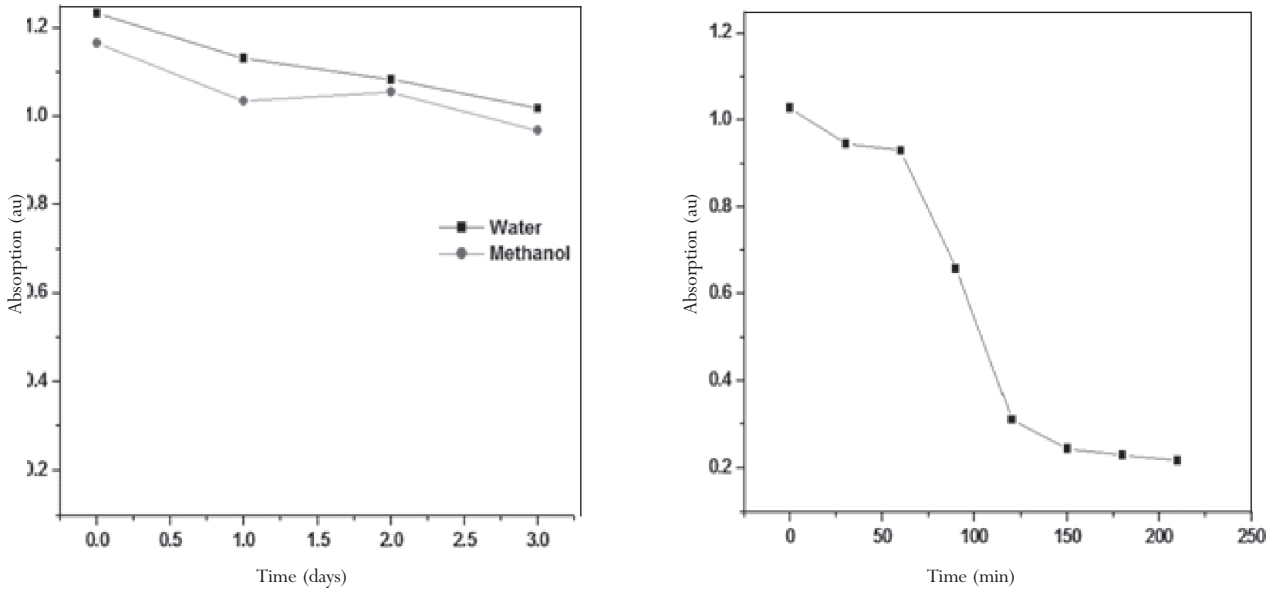

Fig. 6. Solubility of the starch granules in methanol, water (left) and DMSO (right) with respect to time

Acid hydrolysis

The acid hydrolysis pattern of the starch granules is depicted in Fig. 5. Starch granules from the *C. esculenta* was hydrolyzed for 15 days with 2.2 M HCl at 35°C and then the reducing sugar was estimated as per the standard method (Fig.5). The extent of hydrolysis of the starches during initial days (degradation of amorphous regions) was obtained followed by the degradation of crystalline region. Differences in the rate of acid hydrolysis of the starch might be attributed to interaction in relation to amorphous and crystalline region and composition of starch (phosphate content and amylose/amylopectin ratio) (Gunaratne and Hoover, 2002). In B-type starches, α (1→6) branches are located mainly in the amorphous region and thus very susceptible to acid hydrolysis; whereas, in A-type starches, α (1→6) branches are located in the crystalline region and resistant to hydrolysis by H_3O^+. The latter might be the cause of slow initial degradation rate. On day 15, a degradation percentage of 42.27% was obtained during the study.

Gelatinization parameter

As obtained from DSC analysis results the gelatinization parameter of the starch was found to be T_0 = 79.7°C, T_p = 88.1°C, T_c = 95.4°C. The T_c - T_0 = 15.7°C and enthalpy of gelatinization was found to be 13.1 J g^{-1}. The data represents the average of three replications.

Solubility and DPPH scavenging assay

The solubility of the starch granules in three different solvents is necessary for its industrial application. As shown in Fig.6, the starch was highly soluble in DMSO as compared to methanol and water. Out of the other two solvents, methanol showed slightly higher solubility than water (Fig. 6). The trend of solubility observed in DMSO (Fig. 6) indicated that there was an exponential increase in the solubility of starch granules from 50-100 min. But the solubility of starch granules in water and methanol was slow and gradual.

The DPPH scavenging potency of the granules is depicted in Fig. 7. In the case of methanol and water solubilized starch the scavenging was investigated for three days during which DPPH scavenging trend (Fig. 7a and b) was not found to follow the solubility trend of the granules in the solvents. In the case of methanol solvent solubilized starch granules the scavenging was observed in the order of day 3 > day 1 > day 2 (for concentration of 0.2-0.8 mg ml^{-1}) which even become day 1 > day 3 > day 2 at concentration 1.0 mg ml^{-1}. In the case of water solubilized starch granules, the DPPH scavenging order was day 3 > day 1 > day 2 for concentration of 0.2 mg ml^{-1} and 0.4 mg ml^{-1}. At higher concentration the water solubilized starch granules show saturation as the reaction was carried out in heterogeneous system

(solid-liquid) in part and showing finally the order of scavenging as day 1> day 2> day 3 at concentration of 1.0 mg ml^{-1}.

Looking into the scavenging orders it might be the absorption phenomenon rather than scavenging alone which decreases the DPPH availability. The absorption phenomenon coincides with the solubility (Fig. 6) which was also evidence of insoluble proportions. The solubility of starch granules in water was found to be less as compared to that of methanol with a slight difference (Fig. 6) coinciding to that of DPPH scavenging (Fig.7a and b) was found to be more in water suggesting more granular mass was available for absorption of the free radicals. As the soluble starch got their saturation at an applied concentration of 0.4 mg ml^{-1} (Fig. 7c) so day 2 methanol and water solubilized granules have lowest potential as compared to day 1 and 3 samples. The day 2 starch do not have enough solubilized starch like day 3 to scavenge properly with DPPH free radicals as well as they had a scarcity of insoluble part to absorb like day 1 samples (Fig.7a and b). Looking into this anomaly it can be suggested that to prove the absorption and then scavenging phenomenon of starch granules further investigation of the assay was needed. This has to be carried out with soluble supernatant as well as precipitated starch granules at different time intervals in the investigated solvents.

Swelling power

Swelling power of the starch granules at room temperature was found to be 81.99±4.32%. The swelling power suggests that the granules have good water holding capacity and are suitable for use in food thickener and oral re-hydration composite preparation.

Conclusion

The starch is considered as one of the strategic materials of the future. At present the corn and potato starches are dominant

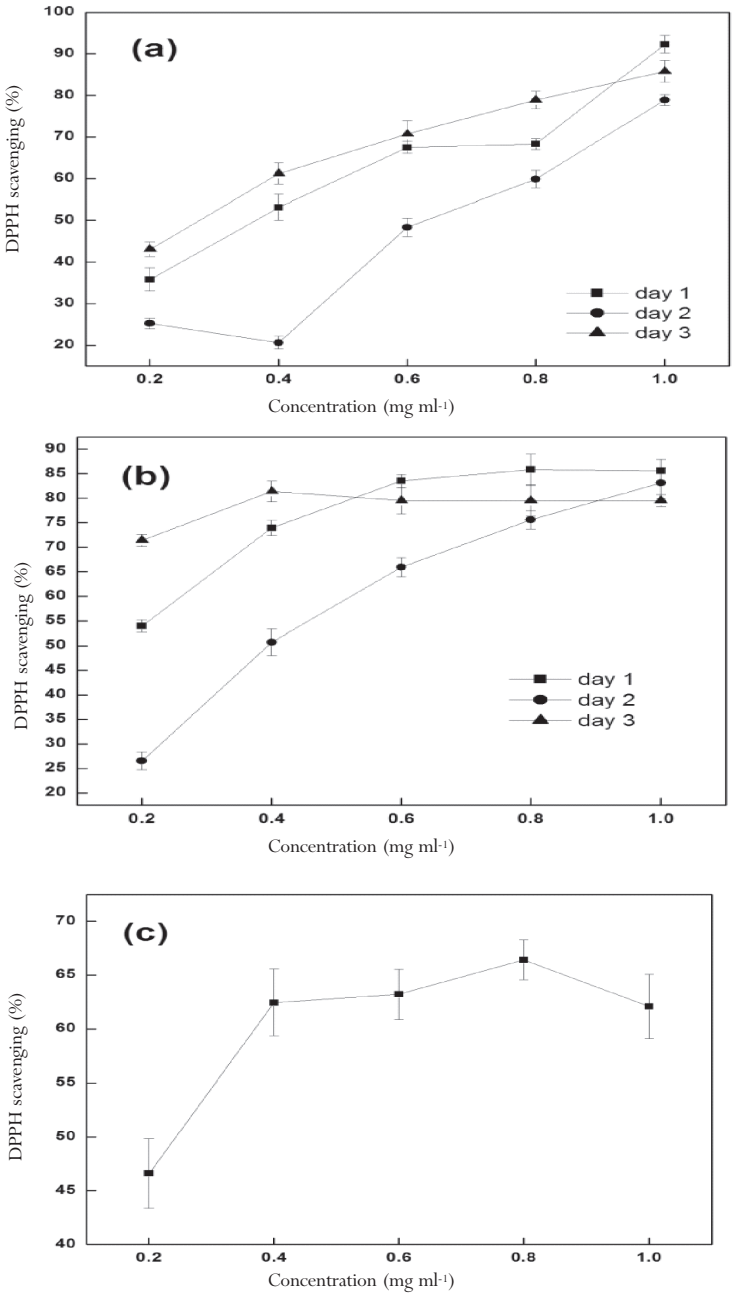

Fig. 7. DPPH scavenging of the starch granules with respect to time and concentration (a) methanol, (b) water and (c) DMSO (not time dependent)

in the market. There is an increasing demand for cheap and alternate source of starch. The present investigation was carried out with the objective to evaluate the food as well as non food application potential of starch with the hope of providing scientific ground to the delicious and popular edible taro species.

The small sizes of the starch granules suggests that they are suitable for use in food and textile industry with minimum processing. The higher crystalline value as compared to earlier report for *Colocasia*

species suggests that the starch granules will be slightly susceptible to enzymatic and acid hydrolysis and they are suitable for use in material research like film and composite preparations. The FT-IR analysis of the starch granules suggests that the isolated starch granules were pure enough for use in industrial processes. The amylose leaching with respect to heat treatment suggests that the rate of leaching increases above 80°C. Therefore to leach out amylose at a faster rate for any industrial process heat treatment above 80°C is needed. The acid hydrolysis of 42.27% after 15 days of treatment suggests that the granules can easily be converted into sugar supplements with acid treatment. The solubility in DMSO was very high as compared to water and methanol. Other parameters like gelatinization enthalpy (13.1 $J.g^{-1}$), solubility in DMSO, water and methanol, antioxidant activity and swelling power (81.99± 4.32%) suggests the suitability of the starch granules for use in food processing industry as supplement, ingredient of packaging material and food thickener etc.

Acknowledgement

Authors acknowledge the Department of Biotechnology for providing fund for the purchase of chemicals for the present investigation. Authors are also thankful to National Medicinal Plant Board for providing financial assistance.

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