



Effect of Ultrasound and Cellulase Pre-Treatment on Extractability and Properties of Taro Starch

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Abstract

Ultrasound and cellulase pre-treatment was applied for the extraction of starch from taro tubers and their effect on yield and properties of the extracted starch was studied. Ultrasonication time was varied at three levels of 0, 10 and 20 min whereas cellulase concentration was varied at two levels of 0 and 200 U/100 g tuber. It was observed that higher yield was obtained with ultrasonication as well as cellulase treatment. When both the treatments were combined, yield achieved was much higher compared to conventional extraction. Highest yield of 19.36% was obtained when ultrasonication was carried out for 20 min and was then treated with cellulase at concentration of 200 U/100 g tubers. The functional properties was comparable to conventionally extracted starch when cellulase treatment alone was carried out. The swelling and solubility increased with ultrasonication and was time dependent. Clarity of the starch pastes improved with cellulase treatment and was negatively affected by ultrasonication. Maximum clarity was observed for starch extracted by cellulase treatment only. Freeze-thaw stability of the starch pastes improved for ultrasonically extracted starch and was not affected by cellulase treatment. Pasting properties were not much affected by ultrasonication for 10 min and cellulase treatment, but when ultrasonication time was increased to 20 min, the peak and final viscosities were found to be significantly increased. Higher yield of starch with better properties like improved freeze-thaw stability, high paste viscosity and swelling and better clarity can be obtained from taro tubers by combined pre-treatment with ultrasound and cellulase, which will help in the commercial exploitation of this underutilized tuber.

Key words: Taro tuber, starch extraction, ultrasonication, enzyme pre-treatment, properties.

Introduction

Taro is a tropical tuber crop and consumed throughout the tropical and sub-tropical regions of the world as a root and leaf vegetable. The crop is underutilized and sold in markets as raw vegetable. The commercial potential of this crop is high in tropical and sub-tropical regions where cereal production is not feasible. Taro corms have high pre-harvest and post-harvest losses. The losses can be minimized if the corms can be converted into non-perishable forms by drying or extracting the starch and other components. (Pérez et al., 2005). Taro tubers contain 70–80% starch on dry weight basis and the starch granules are small in size. The production of taro in North-Eastern region of India is abundant. It is grown in an

unplanned manner by small farmers and is commercially under exploited. *Panchamukhi* is a promising variety of taro from Eastern and North-Eastern India and the productivity is also high, about 45.9 t ha⁻¹ (ICAR, 2007).

The starch granules are embedded within the cells in roots and tubers, which are enclosed by the cell walls comprising cellulosic materials (Rahman and Rakshit, 2004). Extraction of starch from roots and tubers are carried out by mechanically disintegrating the cell wall and then washing out of the starch granules by water (Kallabinsiki and Balagopalan, 1994; Daiuto et al., 2005). These mechanical methods are energy intensive and damage the starch granules to such an extent that it might adversely affect the properties of the starch. Enzymatic methods

have been used by several researchers for the recovery of starch from roots and tubers (Gayal and Hadge, 2003; Padmanabhan et al., 1993; George et al., 1991). Enzymatic methods also have their own disadvantages. They are time consuming and also requires large amount of enzymes which makes the process costly. If mechanical methods can be combined with enzymatic method, then the incubation time and amount of enzyme required can be reduced. Advanced methods like ultrasound, which uses lesser energy, can be used to replace conventional mechanical methods. But, the duration of the ultrasound application has to be controlled in such a way that it does not adversely affect the properties of the starch granules.

Several investigators have used ultrasound for increasing the recovery of starch from cereals like maize (Zhang et al., 2005; Benmoussa and Hamaker, 2011) or to modify the properties of isolated starch (Chan et al., 2010; Luo et al., 2008; Zheng et al., 2013). Reports on the effect of combination of ultrasound with enzymes for starch extraction are rare. Therefore, the objective of the present study was to investigate the effect of ultrasound and cellulase pre-treatments, and their combinations on yield and properties of taro starch.

Materials and Methods

Starch isolation using ultrasound and enzymes

Tubers of *Panchamukhi* Taro (*Colocasia esculenta* var. *antiquorum*) were collected from an agricultural farm near Tezpur University, Assam, India. Tubers were washed with tap water, peeled and cut into cubes of approximately 1 cm. Hundred grams of cubes were weighed and ground using a laboratory blender for two minutes by mixing in 100 ml of water. Ultrasound was applied to the suspension using an ultrasonic processor of 30 kHz and 100 W (UP100H, Hielscher, Teltow, Germany). After ultrasonication, the slurry was subjected to enzymatic treatment using cellulase (100 U per 100 g tuber) from *Aspergillus niger* (Sigma-Aldrich Corp., St. Louis, USA) with an activity of 0.3 U/mg. The slurry was incubated at 45°C for 2 h in an incubator shaker. After incubation the suspension was filtered through a double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min in a refrigerated centrifuge at 20°C. The sediment was dried at 45°C for 24 h in hot air drying oven.

Yield was obtained by calculating the amount of pure starch (dry basis) recovered from 100 g of fresh taro sample.

Chemical analysis and amylose content

Moisture and fibre content for all the isolated starches were determined by AOAC methods (AOAC, 1990). Starch content was determined by acid hydrolysis method using perchloric acid which hydrolysed the starch to glucose and dehydrated it to hydroxymethyl furfural which was then measured by Anthrone reagent (HiMedia, Mumbai, India) (Sadasivam and Manickam, 2011). The starch content of the taro tubers were 21.45 ± 0.86 g (n=5) per 100 g fresh tuber. Protein content ($N \times 6.5$) was determined by Kjeldahl method (AACC, 1990). The ash and fat contents were determined by AOAC methods (AOAC, 1990). The amylose content was determined by colorimetric method (McGrance et al., 1998). The standard curve was prepared using pure potato amylose type III (HiMedia, Mumbai, India).

Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Singhal and Kulkarni (1990). Starch (5% dry basis) was heated in distilled water at 95°C for 30 minutes with constant stirring. Ten ml of paste was transferred to pre-weighed centrifuge tubes. This was subjected to alternate freezing and thawing cycles (22 h freezing at -20°C followed by 2 h thawing at 30°C) for 3 days i.e. 3 freeze-thaw cycles, centrifuged at 5000 × g for 10 minutes in a refrigerated centrifuge at 20°C and the percentage syneresis was determined as weight of supernatant to the weight of paste.

Pasting properties

Pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 (Newport Scientific, Warriewood, Australia). Viscosity profiles were recorded using 12.5% starch slurry in distilled water (total weight 28 g). A heating and cooling cycle of 13 min 30 s was used where the samples were heated from 50°C to 95°C in 5 min, held at 95°C for 2 min, cooled from 95°C to 50°C in 4 min and held at 50°C for 2 min 30 s. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

Clarity of starch pastes

Aqueous starch suspension containing 1% starch was prepared by heating 0.2 g starch in 20 ml water in shaking

water bath at 90°C for 1 h. The starch paste was cooled to room temperature and the transmittance was measured at 640 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Bangalore, India).

Swelling power and solubility

Swelling power and solubility of the starches were determined by modified method of Torruco-Uco and Betancur-Ancona (2007). Starch (0.5 g) was dispersed in 20 ml of distilled water in a pre-weighed 50 ml centrifuge tube and kept in a shaking water bath at 90°C for 30 min. The suspension was then centrifuged at 12,000 × g for 10 min. The supernatant was carefully decanted in a Petri dish and dried at 103°C for 12 h. After decantation the weight of swollen granules were taken. The swelling power and percentage solubility were calculated using the following formulas:

$$\text{Swelling Power} = \frac{\text{Weight of swollen granules}}{\text{Weight of sample} - \text{Weight of dissolved starch}} \times 100$$

$$\% \text{ Solubility} = \frac{\text{Weight of dried starch in Petri dish}}{\text{Sample weight}} \times 100$$

Analysis of data

A 3 × 2 full factorial design was employed to study the effect of ultrasound and cellulase treatment on yield of taro starch. The factors were ultrasonication time (0, 10 and 20 min) and concentration of cellulase (0 and 200 U/100 g fresh tuber). Properties of the starches were determined. Duncan's multiple range test was used to determine the statistical difference as mentioned above.

Results and Discussion

Chemical composition and amylose content

The data for chemical composition and amylose content of the various starch samples are presented in Table 1. It can be observed that the moisture, protein, fat and ash contents did not vary much among the different samples. The fibre content was significantly lower in starches treated with cellulase and was not affected by ultrasound treatment. When ultrasound treatment was combined with cellulase treatment, fibre content significantly decreased in the isolated starch, which further decreased with increase in ultrasonication time. This implies that cellulase is able to breakdown the cellulose fibres in the cell walls of the tuber which resulted in high recovery starch with more purity and less fibre content (Sit et al., 2014a). When ultrasound was applied with cellulase treatment partial breakdown

of the cell wall took place which made the substrate i.e. cellulose readily accessible to cellulase. The purity of the isolated starch was found to be correlated with fibre content, the lower the fibre content the purer the isolated starch.

The amylose content of the isolated starch samples were found to be affected by ultrasound treatment and were not affected by cellulase. Amylose content increased with ultrasonication time. This might be attributed to the partial breakdown of starch granules by the forces generated due to ultrasonication. The more the ultrasonication time, more was the breakdown and higher was the amylose content of the isolated starch.

Yield of starch

Table 1 shows the yield of starches for different treatment conditions. Yield of starch significantly increased with cellulase treatment. Yield also increased with ultrasound treatment, but increasing the time of ultrasonication did not vary the yield significantly. When ultrasound was combined with cellulase treatment, significantly higher yield of starch was obtained. For the combined treatment, it was observed that increasing the time of ultrasonication had significant effect on starch yield. The reason for lower yield with ultrasonication only, might be attributed to the fact that ultrasound was able to partially breakdown the cell wall components and not release much of the starch. When ultrasound was combined with cellulase treatment, the partial breakdown of the cell wall components resulted in the substrates being readily accessible by the enzymes thereby degrading more amount of cellulose (Sit et al., 2014a; Wang and Wang, 2004). Highest yield of starch was achieved when ultrasonication was carried for 20 min followed by cellulase treatment.

Clarity of the starch pastes

It can be seen from Fig. 1 that clarity of the starch pastes was more for the starches isolated by cellulase and those isolated by combined treatments. This might be attributed to the lower fibre content of the starches isolated by cellulase treatment. The clarity of the starch pastes isolated by ultrasound resulted in lower clarity compared to native starch solution. When ultrasound was applied for extraction some impurities might get embedded within the starch granules due to the physical effect of ultrasound, and when a solution is made from such starches, the clarity of the resulted solution decreased.

Table 1. Chemical composition, amylose content and yield of starch at different experimental conditions

Sl. No.	Samples	Cellulase Concentration, U/100g tuber	Ultrasound time, min	Moisture content, %	Protein content, % dry basis	Fat content, % dry basis	Ash content, % dry basis	Fibre content, % dry basis	Starch content, % dry basis	Amylose content, % total starch	Yield of starch %
1	S ₁	0	0	8.43±0.19 ^b	0.32±0.03 ^b	0.11±0.02 ^a	0.06±0.02 ^{ab}	0.29±0.03 ^a	95.39±0.23 ^c	17.93±0.26 ^c	13.57±0.41 ^c
2	S ₂	100	0	8.78±0.33 ^{ab}	0.32±0.04 ^b	0.12±0.02 ^a	0.07±0.01 ^b	0.17±0.02 ^b	96.59±0.72 ^b	18.31±0.58 ^{bc}	16.63±0.45 ^c
3	S ₃	0	10	8.69±0.27 ^{ab}	0.33±0.04 ^b	0.11±0.04 ^a	0.07±0.02 ^{ab}	0.28±0.04 ^a	95.43±0.39 ^c	18.50±0.22 ^{abc}	14.63±0.37 ^d
4	S ₄	0	20	8.58±0.15 ^{ab}	0.28±0.04 ^b	0.11±0.03 ^a	0.09±0.02 ^a	0.26±0.01 ^a	95.68±0.09 ^c	18.95±0.32 ^{ab}	14.92±0.44 ^d
5	S ₅	100	10	8.55±0.16 ^{ab}	0.36±0.06 ^b	0.14±0.02 ^a	0.10±0.02 ^a	0.10±0.02 ^c	96.68±0.61 ^b	18.54±0.18 ^{abc}	18.81±0.67 ^b
6	S ₆	100	20	8.93±0.26 ^c	0.47±0.06 ^c	0.14±0.02 ^a	0.07±0.01 ^{ab}	0.08±0.02 ^c	97.51±0.10 ^b	19.14±0.33 ^a	20.13±0.07 ^a

^{a)} Values reported as Mean ± Std. Dev. of three replications.

^{b)} Means followed by same small letter superscripts within a column are not significantly different ($p < 0.05$)

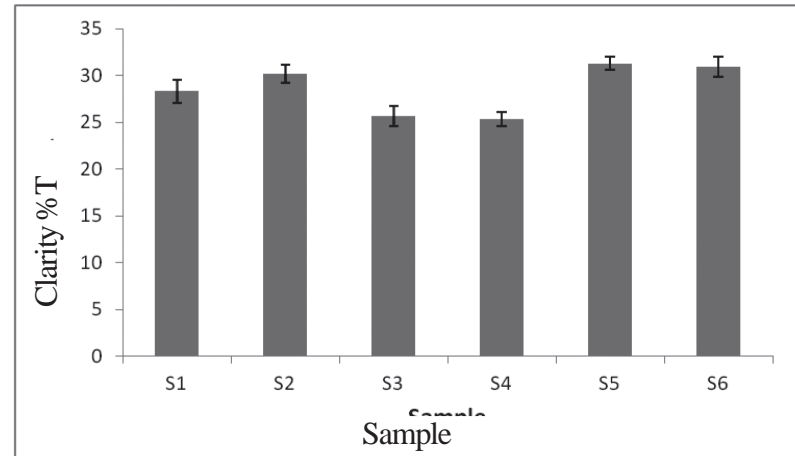


Fig. 1. Paste clarity of starches extracted under different conditions

Swelling power and solubility

The solubility and swelling power of the starches are presented in Table 2. The solubility and swelling power were found to be affected by ultrasonication, whereas enzymatic treatment had no significant effect on these parameters. The swelling of the starch granules were more when the ultrasonication time was more. This might be attributed to higher breakdown of the starch structure with time thereby exposing more hydrophilic groups to water and leading to higher water uptake and retention (Jambrak et al., 2010; Tester and Morrison, 1990).

Freeze-thaw stability

The freeze-thaw stability (syneresis) of the starch pastes can be seen in Table 2. It was observed that freeze-thaw stability of the ultrasonically extracted starches were better compared to control and starch isolated by cellulase alone. Ultrasonically extracted starches were observed to be more stable under repeated freeze-thaw cycles. Breakage of starch chains in the amorphous region caused extensive reordering of the chain segments due to ultrasonic treatment (Luo et al., 2008; Czechowska-Biskup et al., 2005). A similar observation on freeze-thaw behaviour of ultrasonically treated maize starch was made by Luo et al. (2008).

Table 2. Solubility, swelling and freeze-thaw stability of starch pastes at different experimental conditions

Treatment ^{a),b)}	Solubility at 90°C, %	Swelling power at 90°C, g/g	% Syneresis after 3 freeze-thaw cycles
S1	20.22±0.84 ^b	14.23±0.36 ^b	34.21±1.23 ^a
S2	20.46±0.12 ^{ab}	14.19±0.21 ^b	33.95±0.98 ^a
S3	20.86±0.85 ^a	14.69±0.68 ^{ab}	26.12±0.65 ^b
S4	20.14±0.56 ^b	15.16±0.28 ^a	25.23±0.87 ^{bc}
S5	20.67±0.29 ^{ab}	14.52±0.48 ^{ab}	25.63±0.36 ^{bc}
S6	20.48±0.29 ^{ab}	14.87±0.37 ^{ab}	24.56±0.84 ^c

^{a)} Values reported as Mean ± Std. Dev. of three replications

^{b)} Means followed by same small letter superscripts within a column are not significantly different ($p < 0.05$)

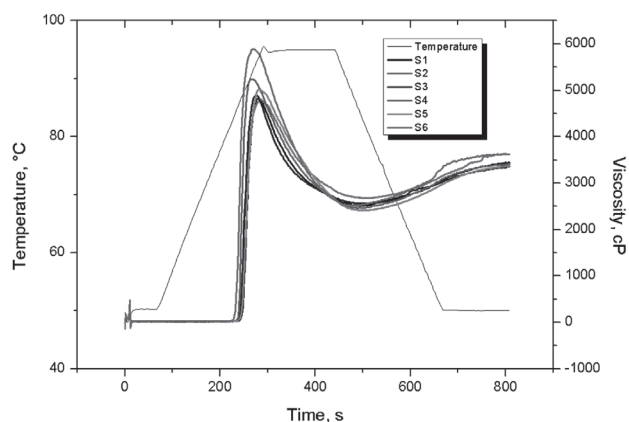


Fig. 2. Pasting profile of starches extracted under different experimental conditions

Pasting properties

The pasting profile of the starches isolated by different methods is shown in Fig. 2. It can be observed that the peak viscosities of the starches extracted by ultrasound treatment were higher than that of control i.e. S1. The peak viscosity was not affected by cellulase treatment. When time of ultrasonication was increased an increase in peak viscosity was noted. When both the treatments were combined, the peak viscosity was found to be more than the individual treatments. Highest peak viscosity was observed for S6 where ultrasonication for 20 min was combined with cellulase treatment. Similar results were obtained for hold and final viscosities. The increase in viscosity with ultrasonication might be attributed to greater disruption of the granule structure for more sonication time, which allowed more water to be absorbed and thereby increasing the peak viscosity (Sit et al., 2014b).

Conclusion

Both ultrasound and cellulase treatment can be used to obtain higher yield of starch from taro tubers. The physical properties like pasting, and freeze-thaw stability of starch were altered due to ultrasonication and were found to be better. Enzyme treatment yielded starch with higher clarity. Therefore, it can be concluded that higher yield of starch with better functional properties can be achieved if ultrasound treatment is combined with cellulase treatment for extraction of starch from taro tubers.

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