Optimization of starch isolation from taro using combination of enzymes and comparison of properties of starches isolated by enzymatic and conventional methods

Nandan Sit · Sankar Chandra Deka · Sudip Misra

Revised: 19 June 2014 / Accepted: 27 June 2014 / Published online: 6 July 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract The optimization of enzymatic starch isolation process from taro tubers using cellulase and xylanase was carried out. The functional properties of starch isolated by optimized enzymatic process were compared with starch isolated by conventional method without the use of enzymes. A central composite rotatable design (CCRD) with four numerical factors was employed to design the experiments. The numerical factors were cellulase concentration (0–100 U/100 g tuber), xylanase concentration (0–100 U/100 g tuber), temperature of incubation (30–50 °C) and incubation time (1–5 h). Statistical analysis showed that the main effects of all the factors were significant on starch yield and effect of cellulase was more significant compared to xylanase. The effectiveness of xylanase in increasing the yield of starch from taro tubers confirmed that xylan is an important component of the cell walls of taro tubers. The optimized condition with maximum starch yield (17.22 %) was obtained when cellulase and xylanase concentration were 299.86 and 300 U/100 g tuber, temperature was 35 °C and incubation time was 2 h. The swelling of the starch granules increased whereas solubility decreased for enzymatic method. The clarity of the starch paste isolated by enzymatic method was found to be better compared to the clarity of starch paste isolated by conventional method. The pasting temperature of the starch paste was slightly higher and viscosity was lower for the starch isolated by enzymatic method. Freeze-thaw stability of the starch paste was also found to be better for the enzymatically isolated starch.

Keywords Cellulase · Xylanase · Taro starch isolation · Optimization · Functional properties

Introduction

Starch is a polysaccharide of glucose linked by α , 1–4 glycosidic bonds. It is the main reserve carbohydrate produced by all green plants. Starch is the most important carbohydrate in human diet and is contained in many staple foods from cereals to roots and tubers. Starches have many industrial applications. Starch is an important ingredient in the food industry. It is also an important raw material for paper, pharmaceutical, textile and cosmetic industries (Daiuto et al. 2005; Wischmann et al. 2007; Nand et al. 2008; Mweta et al. 2008).

Taro (*Colocasia esculenta*) is a tropical tuber crop largely produced for its underground corms and consumed in tropical and sub-tropical areas of the world. The potential of this crop is high in humid and sub humid tropics where cereal production is not suitable (Purseglove 1972). Taro has been reported to have 70–80 % starch with small granules (Jane et al. 1992). Taro starch is used directly in different ways or as a raw material for further processing. It is considered to be easily digestible because of the small sizes of its starch granules; hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk (Benesi et al. 2004; Nip et al. 1997). The in vivo digestibility of taro starch was found to be comparable to corn starch (Moorthy 2002) and was more susceptible to pancreatin hydrolysis compared to other tuber and root starches (Sugimoto et al. 1979). Taro starch has also been used for industrial applications because of its small granule size (Griffin and Wang 1983). It can be used in the preparation of biodegradable polyethylene film (Griffin and Wang 1983; Lim et al. 1992). It can also be used as an entrapping agent for flavoring compounds like vanillin (Zhao and Whistler 1994; Tari and Singhal 2002). It is also suitable for cosmetic formulations like face powder and in dusting preparations that use aerosol dispensing systems (Griffin and Wang 1983).

N. Sit (✉) · S. C. Deka · S. Misra
Department of Food Engineering and Technology,
Tezpur University, Tezpur, Assam 784028, India
e-mail: nandansit@yahoo.co.in

The starch granules present in the roots and tubers are embedded in cellulosic fibres and held together by pectin substrates (Rahman and Rakshit 2004). The high water content and other morphological similarities of tuber crops require a familiar technological process of starch extraction from these crops (Kallabinski and Balagopalan 1994). The starch extraction process from roots and tubers involves of grating the raw material, in order to break vegetal cells and release the starch. This step is followed by passing the fibre through sieves of different mesh sizes and subsequent slurry concentration by decantation or centrifugation (Daiuto et al. 2005). The success of starch extraction from tubers depends on complete rupture of the cell walls and thereby releasing the starch granules (Bergthaller et al. 1999).

The cell walls of raw taro corms contain hemicelluloses like xylan, though it is in small amounts, apart from cellulose (Hussain et al. 1984; Quach et al. 2000), but helps in maintaining the rigidity of the cell walls. Breakdown of both cellulose and hemicelluloses are necessary for recovery of intracellular materials like starch. Enzymatic methods have been used by several researchers to increase the recovery of starch from roots and tubers (Dzogbefia et al. 2008; Gayal and Hodge 2003; Padmanabhan et al. 1993; George et al. 1991). Most of the methods relied on use of cellulases and pectinases, but till now no investigation on the use of xylanase has been reported. Therefore the objectives of the present study were 1) to investigate the effect of cellulase and xylanase on recovery of starch from taro corms and optimize the enzymatic starch isolation process, and 2) to compare the functional properties of starch extracted by enzymatic method with that of conventional method.

Materials and methods

Starch isolation using enzymes

Taro tubers locally known as *Panchamukhi* (*Colocasia esculenta* var. *antiquorum*) was collected from an agricultural farm near Tezpur University, Assam, India. Tubers were washed under tap water, peeled and cut into cubes of approximately 1 cm. 100 g of cubes were weighed and ground using a laboratory blender (Philips HL 1632, India) for 1 min 30 s. The slurry was mixed with 100 ml distilled water and transferred to a 250 ml beaker. The slurry was subjected to enzymatic treatment using cellulase from *Aspergillus niger* (Sigma-Aldrich, 0.3 U/mg) and xylanase from *Thermomyces lanuginosus* (Sigma-Aldrich, 2,500 U/g) and their combination. The slurry was incubated with different concentrations of cellulase and xylanase for varying time at different temperatures as per the experimental design in incubator shaker (CERTOMAT® IS, Sartorius Stedim Biotech, Goettingen,

Germany) at 150 rpm. After incubation the suspension was filtered through double fold cheese cloth and the filtrate was centrifuged at $3,000\times g$ for 10 min. The supernatant was discarded and sediment was washed twice with distilled water. The final sediment was dried at 45 °C for 24 h in hot air drying oven (Oven Universal, JSGW, Ambala Cantt., India) with internal heating and natural convection mode. The dried starch was ground and passed through 100 mesh sieve and kept in air tight plastic containers for further analysis.

Moisture and starch contents of the dried samples were determined to calculate the yield of pure starch. Moisture content was determined by hot air oven method (AOAC 1990) and starch content was determined by acid hydrolysis method using perchloric acid (Sadasivam and Manickam 2011). The starch was hydrolysed to glucose which was then dehydrated to hydroxymethyl furfural. The concentration of glucose was determined by spectrophotometrically measuring the concentration of hydroxymethyl furfural using Anthrone's reagent. The concentration of glucose obtained in percent was converted to percent starch by multiplying by 0.9. The starch content of the taro tubers were 21.96 ± 0.42 g ($n=5$) per 100 g fresh tuber (21.96 % wet basis).

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

Experimental design

A central composite rotatable design (CCRD) with four numerical factors was employed to design the experiments. The numerical factors were cellulase concentration (C), xylanase concentration (X), temperature of incubation (T) and incubation time (t). The cellulase and xylanase concentration were varied from 0 to 100 U/100 g taro tuber, incubation time was varied from 1 to 5 h and temperature was varied from 30 to 50 °C. A total of 30 experiments were performed (Table 1). The 30 experiments were selected on the basis that for CCRD there should be 2^k factorial points (in this case it is 16, core design), 2 k axial or star points (in this case it is 08, outside the core) and several centre points (here it is 06) where k is the number of factors (here it is 04). Six experiments at the centre points of the design were performed to allow the estimation of pure error. All experiments were carried out in a randomized order to minimize the effect of external factors (Wanasundara and Shahidi 1998). All the experiments were carried out in triplicates.

Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Singhal and Kulkarni (1990). 5 % (w/v) starch (db) was heated in distilled water at 95 °C for 30 min with

Table 1 Starch yield for different combinations of experimental conditions

Experiment No.	Time, t, h	Temp, T, °C	Cellulase, C, U/100 g tuber	Xylanase, X, U/100 g tuber	Yield,% ^b
1	1(−2) ^a	40(0)	200(0)	200(0)	14.46±0.21
2	2(−1)	35(−1)	100(−1)	100(−1)	13.56±0.15
3	2(−1)	35(−1)	100(−1)	300(1)	15.67±0.32
4	2(−1)	35(−1)	300(1)	100(−1)	15.99±0.09
5	2(−1)	35(−1)	300(1)	300(1)	17.49±0.23
6	2(−1)	45(1)	100(−1)	100(−1)	13.17±0.11
7	2(−1)	45(1)	100(−1)	300(1)	15.16±0.17
8	2(−1)	45(1)	300(1)	100(−1)	15.48±0.27
9	2(−1)	45(1)	300(1)	300(1)	15.94±0.41
10	3(0)	30(−2)	200(0)	200(0)	16.57±0.18
11	3(0)	40(0)	0(−2)	200(0)	13.99±0.22
12	3(0)	40(0)	200(0)	0(−2)	14.29±0.12
13	3(0)	40(0)	200(0)	200(0)	15.19±0.16
14	3(0)	40(0)	200(0)	200(0)	15.31±0.08
15	3(0)	40(0)	200(0)	200(0)	15.11±0.09
16	3(0)	40(0)	200(0)	200(0)	14.96±0.22
17	3(0)	40(0)	200(0)	200(0)	15.24±0.24
18	3(0)	40(0)	200(0)	200(0)	15.38±0.26
19	3(0)	40(0)	200(0)	400(2)	16.02±0.23
20	3(0)	40(0)	400(2)	200(0)	16.69±0.12
21	3(0)	50(2)	200(0)	200(0)	15.05±0.13
22	4(1)	35(−1)	100(−1)	100(−1)	14.78±0.13
23	4(1)	35(−1)	100(−1)	300(1)	16.02±0.15
24	4(1)	35(−1)	300(1)	100(−1)	16.45±0.24
25	4(1)	35(−1)	300(1)	300(1)	16.61±0.36
26	4(1)	45(1)	100(−1)	100(−1)	14.65±0.25
27	4(1)	45(1)	100(−1)	300(1)	15.54±0.16
28	4(1)	45(1)	300(1)	100(−1)	15.96±0.27
29	4(1)	45(1)	300(1)	300(1)	16.03±0.32
30	5(2)	40(0)	200(0)	200(0)	15.21±0.14

^a Numbers in parentheses correspond to coded values of different levels of factors

^b Values reported as Mean±S. D. of three replications

constant stirring. 10 ml of paste was transferred to pre-weighed centrifuge tubes. The weight of the paste was then determined. 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, centrifuged at 5,000×g for 10 min after each cycle and the percentage syneresis was determined as weight of exudates to the weight of paste.

Pasting properties

The pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 from Newport Scientific, 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 to 95 °C in 5 min, held

at 95 °C for 2 min, cooled from 95 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=PV-HV) and setback viscosity (SV=FV-HV) were recorded from the graph.

Swelling and solubility

The 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 distilled water in a pre-weighed 50 ml centrifuge tubes and kept in 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:

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

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

Clarity of starch pastes

The clarity of the starches was measured following the method described by Sandhu and Singh (2007). 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, India).

Data analysis, optimization and model validation

Design Expert version 8 was used for analysis of data for starch yield and optimization. Experimental data were fitted to a second order polynomial model as follows:

$$Y = \beta_o + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i < j=1}^3 \beta_{ij} X_i X_j$$

Where Y represents the response i.e. starch recovery, β_o is the constant, β_i , β_{ii} and β_{ij} are the regression coefficients and X_i and X_j are the independent variables in coded values.

Significant terms in the model were found by analysis of variance (ANOVA). Model adequacy was checked by lack of fit test, R^2 , predicted R^2 , adequacy precision and predicted residual sum of squares (PRESS). A non-significant ($p > 0.05$) lack of fit, predicted R^2 comparable to fitted R^2 , low PRESS and adequacy precision higher than 4, shows that the model fitted is adequate to predicting (Corzo et al. 2008; Erbay and Icier 2009; Mohapatra and Bal 2010).

Optimization of the extraction process was done using desirability function. In order to determine the accuracy of the model and validate it, the values of the independent factors (cellulase concentration, xylanase concentration, time of incubation and temperature of incubation), were randomly selected within the design space and the actual starch yield obtained were compared with the predicted values using the model. Five experiments were conducted for validation. The factors were randomly selected so that proper validation of the model could be done. If the factors were not randomly selected then the outcome might be biased.

Response surfaces were generated to study the effect of interactions on starch recovery. The functional properties of the starches were determined for optimized condition for enzymatic method and conventionally extracted starches in replicates of three. Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

Results and discussion

Model fitting

The values of starch yield for different experimental combinations are shown in Table 1. Multilinear regression analysis of the data yielded second order polynomial equations for starch yield. Analysis of variance (ANOVA) was performed to determine the significant effects of the process variables on starch yield. Regression coefficients of the different terms in the equations for starch yield in coded factors were obtained (Table 2). Model adequacy was checked by lack of fit test and by considering fitted R^2 , predicted R^2 , PRESS and adequacy precision. A non-significant ($p > 0.05$) lack of fit, predicted R^2 comparable to fitted R^2 , low PRESS and adequacy precision higher than 4, implies that the model fitted is adequate to predicting (Corzo et al. 2008; Erbay and Icier 2009).

It can be observed from Table 2 that the probability (p) values of the model, all the main factors, quadratic term for only temperature (T^2) and interaction of $t \times C$, $t \times X$, and $C \times X$ for the response significant and the model has non-significant lack of fit ($p > 0.05$) with p -value of 0.1404, which is good, and also the adequacy precision for the model was more than 4. The R^2 value of the model was 0.97, whereas the adjusted R^2 (0.95) and predicted R^2 (0.86) were comparable indicating that the model fitted provided appropriate approximation of the true process.

The final equations in terms of coded factors are as follows:

$$\begin{aligned} \text{Starch yield, } Y = & 15.20 + 0.21t - 0.32T + 0.70C \\ & + 0.49X - 0.05t^2 + 0.19T^2 + 0.08C^2 \\ & + 0.03X^2 + 0.08t \times T - 0.20t \times C - 0.23t \\ & \times X - 0.10T \times C - 0.10T \times X - 0.25C \times X \end{aligned} \quad (1)$$

Whereas, the final equation in terms of actual factors is as follows:

$$\begin{aligned} \text{Yield, } \%Y = & 22.20 + 0.72t - 0.65T + 0.02C \\ & + 0.02X - 0.05t^2 + 0.01T^2 + 0.02t \times T \end{aligned} \quad (2)$$

From the coefficients in Eq. 1 it can be observed that temperature had a negative effect on the starch yield and all

Table 2 Analysis of variance (ANOVA) for fitted model of starch yield

Source	Sum of Squares	DF	Mean Square	F-Value	p-value
Model	25.45	14	1.82	37.83	<0.0001 ^a
t	1.08	1	1.08	22.38	0.0003 [*]
T	2.46	1	2.46	51.15	<0.0001 [*]
C	11.76	1	11.76	244.75	<0.0001 [*]
X	5.88	1	5.88	122.39	<0.0001 [*]
t ²	0.07	1	0.07	1.42	0.2527
T ²	1.03	1	1.03	21.47	0.0003 [*]
C ²	0.16	1	0.16	3.34	0.0877
X ²	0.03	1	0.03	0.52	0.4816
t×T	0.10	1	0.10	2.13	0.1650
t×C	0.67	1	0.67	13.99	0.0020 [*]
t×X	0.86	1	0.86	17.81	0.0007 [*]
T×C	0.16	1	0.16	3.41	0.0845
T×X	0.16	1	0.16	3.33	0.0880
C×X	1.02	1	1.02	21.23	0.0003 [*]
Residual	0.72	15	0.05		
Lack of Fit	0.61	10	0.06	2.72	0.1404
Pure Error	0.11	5	0.02		
Correlation Total	26.17	29			
R ²	0.97				
Adjusted R ²	0.95				
Predicted R ²	0.86				
Adequate Precision	26.21				
PRESS	3.67				

^a* Terms significant at $p < 0.05$

the other factors had a positive effect. From Eqs 1 and 2 it could be seen that effect of all the main factors were significant on starch yield.

Optimization of starch isolation process from taro tuber

Optimization of starch isolation process from taro tubers using cellulase and xylanase was done to obtain maximum yield of starch. There are several methods of optimization. When there are few process variables, one method is to overlay the contour plots i.e., graphical method. Another method of solving the optimization problem is by desirability function where all the responses are combined into one measurement (Park and Park 1997). In the present study optimization was done using desirability function that aimed at finding the levels of process variables, which would give maximum yield of starch. One possible solution with highest desirability was selected (Table 3). The optimum condition with highest yield of 17.22 % was obtained when cellulase and xylanase concentration were 299.86 and 300 U/100 g tuber respectively, temperature was 35 °C and incubation time was 2 h.

Validation of the model and optimization result

The model was validated for the four independent variables by randomly selecting the values of the variables within the design space, including the optimization result and comparing the actual values with predicted values from the model. The residual and percentage error between the predicted and actual values were calculated and presented in Table 4. It was observed that the percentage errors between the actual and predicted values for starch yield varied from 1.06 to 6.42 % which is within the acceptable range (Qi et al. 2009). As the actual and predicted values of starch yield were close enough the model is successfully validated.

Effect of interaction of various factors on starch yield

The variation in starch yield with time and temperature is shown in Fig. 1a). Starch yield increased with incubation period but decreased as the temperature was increased. Shah (2007) also reported similar findings for incubation period in extraction of litchi juice using enzymes and Guan and Yao (2008) observed similar changes with temperature for extraction of protein from oat bran using enzymes. The decrease in

Table 3 Results of optimization of starch recovery by desirability function

Sl. No.	Time, h	Temperature, °C	Cellulase, U/100 g tuber	Xylanase, U/100 g tuber	Yield, %	Desirability
1	2.00	35.00	299.86	300.00	17.22	0.94

yield of starch with increase in temperature might be attributed to the loss of activity of the enzymes at higher temperature and resulted in lower breakdown of the cell wall components and thereby releasing lower amount of starch. Another possible reason might be that, at higher temperatures some amount of starch might become solubilised in the water and could not be recovered during centrifugation.

Starch yield increased with increase in concentration of cellulase/xylanase for all incubation periods [Fig. 1b and c]. When the concentration of cellulase or xylanase was high, significant variation in starch yield was not observed with increase in incubation time. But, when the concentrations of the enzymes were low, starch yield increased with increase in incubation time, indicating that less time is required to hydrolyze the cell wall components when concentration of the enzymes were high. The starch yield remained almost constant with change in incubation time when the concentrations of the enzymes were high. However, it was found to increase with increase in concentration of enzymes when incubation time was constant. Similar findings were reported for cassava starch yield by Dzogbefia et al. (2008) using pectinase enzyme and Shah (2007) for litchi juice extraction. Starch yield was found to increase with increase in concentration of the enzymes. This was observed regardless of incubation time. This shows that higher yield of starch similar to that when enzyme concentrations were high, cannot be achieved by keeping low concentration of the enzymes even when the incubation time is increased. This might be due to inhibition of the enzymes by the degradation products of the enzymes i.e. glucose and xylose. When the enzymes were present at lower concentrations most of the enzymes might be inhibited by the products and therefore could never give similar yield comparable with higher concentration of enzymes even when the incubation time is increased.

From Fig. 1 d and e it can be observed that starch yield decreased with increase in temperature. At lower temperature, wide variation in starch yield was observed with change in concentration of enzyme as compared to when the temperature was higher. This might be attributed to inactivation of the enzymes or solubilization of some starch at higher temperatures thereby decreasing the yield.

It has already been observed that starch yield increased with increase in concentration of both the enzymes and combination of the two enzymes significantly increased the yield of starch from the tubers [Fig. 1f]. The effectiveness of xylanase in increasing the recovery of starch from taro tubers clearly indicates that xylan is also an important component of the cell walls of taro tubers, and degradation of xylan is necessary for releasing starch granules from the cells of taro. These observations support the optimization result obtained, i.e. starch yield was maximum when the concentrations of the both the enzymes were high, and incubation time and temperature were low.

Functional properties of starch isolated by enzymatic and conventional methods

The data for swelling, solubility and clarity of the starch pastes are presented in Table 5 and pasting properties is presented in Table 6 for starches isolated by enzymatic and conventional methods. It was observed that starch isolated by enzymatic method swelled significantly more as compared to conventional method. On the contrary, starch isolated by conventional method had higher solubility compared to enzymatic method. Dzogbefia et al. (2008) reported lower swelling and higher solubility for cassava starch using pectinase as opposed to the present finding, and the variation might be due to the use of different kind of enzyme. Correia et al. (2012) and Puchongkavarin

Table 4 Validation of model and optimization result

Sl. No.	Time, h	Temperature, °C	Cellulase, U/100 g tuber	Xylanase, U/100 g tuber	Predicted yield, %	Actual Yield, %	Residual	Error, %
1.	2	40	200	100	14.25	14.9	0.65	4.36
2.	3.5	35	100	225	15.32563	15.49	0.16	1.06
3.	4	42.5	250	100	15.4775	16.54	1.06	6.42
4.	2.5	37.5	150	150	14.5225	13.66	-0.86	6.31
5	2.00	35.00	299.86	300.00	17.22	17.41	0.19	1.09

Fig. 1 Variation in starch yield due to interaction of (a) temperature and incubation time, (b) concentration of cellulase and incubation time, (c) concentration of xylanase and incubation time, (d) concentration of cellulase and temperature, (e) concentration of xylanase and temperature, and (f) concentration of xylanase and cellulase

et al. (2005) also observed similar increase in swelling for chestnut starch and rice starch respectively with

enzymatic methods. The higher swelling and lower solubility in the present investigation might be attributed to

Table 5 Swelling, solubility and clarity of starch samples isolated by conventional and enzymatic methods

Isolation method ^{a, b}	Swelling, g/g	Solubility, %	Clarity, %T
Conventional	13.32±0.43 b	20.21±0.12 a	32.67±1.21 b
Enzymatic	14.95±0.31 a	19.08±0.27 b	38.94±1.74 a

^a Values reported as Mean±S. D. of three replications

^b Means followed by same small letters within a column are not significantly different ($p>0.05$)

loss of amylose in enzymatic method which might have leached out during incubation. Amylose hinders swelling and contributes to the soluble portion of the starch and loss of amylose affected the swelling and solubility.

The clarity of the starch pastes extracted by enzymatic method was found to be more as compared to conventional method. The increase in clarity of the starch pastes due to enzymatic treatment might be attributed to the breakdown of the cell wall components like cellulosic and hemicellulosic fibres into soluble fragments which were removed during centrifugation or else it can contribute to the impurities of the isolated starch.

The pasting properties of the starches isolated by the two methods evinced no significant differences in pasting temperature, PV, HV, FV, SV and BV values, although the pasting temperature was slightly higher and viscosities were lower in the starch pastes isolated by enzymatic method. This might be due to loss of amylose in enzymatic method which might have increased the viscosity of the starch pastes. Dzogbefia et al. (2008) also did not observe significant differences in pasting properties in cassava starch isolated by enzymatic and conventional methods. The results indicate that enzymatic treatment has no adverse effect on the pasting properties of the starch which could be used to achieve higher recovery of starch.

The freeze-thaw stability of the starch gels were evaluated up to three freeze-thaw cycles and are shown in Fig. 2. The freeze-thaw stability of the starch pastes isolated by enzymatic method was more stable as the % syneresis was lower and the differences were less among the three freeze-thaw cycles. Differences in freeze-thaw stability among different types of starches might be due to a variety of factors, most notably,

Fig. 2 Freeze-thaw behaviour of starches isolated by conventional and enzymatic methods^a. ^aColumns separated by same small letters within a cycle are not significantly different ($p>0.05$)

amylose content (Baker and Rayas-Duarte 1998). The better freeze thaw stability of the enzymatically isolated starch might be attributed to loss of amylose during incubation, and lower amylose corresponds to lower retrogradation tendency, thereby lowering syneresis (Ciacco and D'Appolonia 1977).

Conclusion

The study revealed that higher yield of starch from taro tubers could be achieved using cellulase and xylanase. However, combination of both the enzymes yielded significantly higher amount of starch. Effectiveness of xylanase indicated that xylan is an important component of the cell walls of taro tubers and removal of xylan from the cell walls of taro is necessary for achieving higher yield of starch from taro. The highest yield of starch was achieved when both time and temperature were low and concentrations of the enzymes were high. The study also indicated that certain modification of the properties of starch is possible due to treatment with enzymes. The clarity, swelling and freeze-thaw stability of the enzymatically isolated starch increased, whereas the solubility and viscosity decreased slightly, but the changes were not major. Thus, it can be concluded that higher yield of starch from taro tubers could be obtained using enzymes with only slight modifications in the properties of native starch.

Table 6 Pasting properties starch isolated by conventional and enzymatic methods

Isolation method ^{a, b}	Pasting Temperature, °C	Peak Viscosity, cP	Hold Viscosity, cP	Final Viscosity, cP	Breakdown Viscosity, cP	Setback Viscosity, cP
Conventional	84.4±0.30 a	4,333±120 a	2,218±134 a	2,868±97 a	2,115±89 a	650±34 a
Enzymatic	84.6±0.40 a	4,272±147 a	2,126±156 a	2,697±108 b	2,146±67 a	571±53 b

^a Values reported as Mean±S. D. of three replications

^b Means followed by same small letters within a column are not significantly different ($p>0.05$)

Acknowledgments The authors gratefully acknowledge the financial help received from Ministry of Food Processing Industries, Government of India, New Delhi for carrying out the research work.

References

- AOAC (1990) Method 935.29 (Moisture). Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington
- Baker LA, Rayas-Duarte P (1998) Freeze-thaw stability of amaranth starch and the effects of salt and sugars. *Cereal Chem* 75:301–307
- Benesi IRM, Labuschagne MT, Dixon AGO, Mahungu NM (2004) Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments. *J Sci Food Agric* 84:1381–1388
- Berghaller W, Witt W, Goldau H-P (1999) Potato starch technology. *Starch-Starke* 51:235–242
- Ciacco CF, D'Appolonia BL (1977) Characterization of starches from various tubers and their use in bread making. *Cereal Chem* 54:1096–1107
- Correia PR, Nunes MC, Beirão-da-Costa ML (2012) The effect of starch isolation method on physical and functional properties of Portuguese nuts starches. I. Chestnuts (*Castanea sativa* Mill. var. Martainha and Longal) fruits. *Food Hydrocoll* 27:256–263
- Corzo O, Bracho N, Va'squez AA, Pereira A (2008) Optimization of a thin layer drying process for coroba slices. *J Food Eng* 85:372–380
- Daiuto É, Cereda M, Sarmiento S, Vilpoux O (2005) Effects of extraction methods on yam (*dioscorea alata*) starch characteristics. *Starch-Starke* 57:153–160
- Dzogbefia VP, Ofosu GA, Oldham JH (2008) Physicochemical and pasting properties of cassava starch extracted with the aid of pectin enzymes produced from *Saccharomyces cerevisiae* ATCC52712. *Sci Res Essays* 9:406–409
- Erbay Z, Icier F (2009) Optimization of hot air drying of olive leaves using response surface methodology. *J Food Eng* 91:533–541
- Gayal SG, Hadge GB (2003) Isolation of starch from potato using fungal enzyme. *Indian J Microbiol* 43:171–173
- George M, Padmaja G, Moorthy SN (1991) Enhancement in starch extractability from cassava (*Manihot esculenta* crantz) tuber through fermentation with a mixed culture inoculum. *J Root Crop* 17:1–9
- Griffin G, Wang JK (1983) Industrial uses. In: Wang JK (ed) Taro: a review of *Colocasia esculenta* and its potentials. University of Hawaii Press, Honolulu, p 301
- Guan X, Yao H (2008) Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology. *Food Chem* 106:345–351
- Hussain M, Grenville N, Nele RJ (1984) Composition and nutritive value of cormels of *Colocasia esculenta* (L.) Schott. *J Sci Food Agric* 35:1112–1119
- Jane J, Shen L, Chen J, Lim S, Kasemsuwan T, Nip WK (1992) Physical and chemical studies of taro starches and flours. *Cereal Chem* 69:528–535
- Kallabinski J, Balagopalan C (1994) Enzymatic extraction of starch from tropical root and tuber crops. *Acta Horticult* 380:83–88
- Lim S, Jane J, Rajagopalan S, Seib PA (1992) Effect of starch granule size on physical properties of starch-filled polyethylene film. *Biotechnol Progr* 8:51
- Mohapatra D, Bal S (2010) Optimization of polishing conditions for long grain basmati rice in a laboratory abrasive mill. *Food Bioprocess Technol* 3:466–472
- Moorthy SN (2002) Physicochemical and functional properties of tropical tuber starches: a review. *Starch-Starke* 54:559–592
- Mweta DE, Labuschagne MT, Koen E, Benesi IR, Saka JD (2008) Some properties of starches from cocoyam (*Colocasia esculenta*) and cassava (*Manihot esculenta* Crantz.) grown in Malawi. *Afr J Food Sci* 2:102–111
- Nand AV, Charan RP, Rohindra D, Khurma JR (2008) Isolation and properties of starch from some local cultivars of cassava and taro in Fiji. *S Pac J Nat Sci* 26:45–48
- Nip WK, Smith DS, Cash JN, Hui YH (1997) Taro: processing vegetable and technology. Technomic Publishing, Pennsylvania, pp 355–387
- Padmanabhan S, Ramakrishna M, Lonsane BK, Krisnaiah MM (1993) Enzymatic treatment of cassava flour slurry for enhanced recovery of starch. *Food Biotechnol* 7:1–10
- Park SH, Park JU (1997) Simultaneous optimization of multiple response using weighted desirability function. *J Korean Soc Qual Manag* 25:56–68
- Puchongkavarin H, Varavinit S, Berghaller W (2005) Comparative study of pilot scale rice starch production by an alkaline and an enzymatic process. *Starch-Starke* 57:134–144
- Purseglove JW (1972) Tropical crops, In: Monocotyledons. Longman London
- Qi BK, Chen XR, Shen F, Su Y, Wan YH (2009) Optimization of enzymatic hydrolysis of wheat straw pretreated by alkaline peroxide using response surface methodology. *Ind Eng Chem Res* 48:7346–7353
- Quach ML, Melton LD, Harris PJ, Burdon JN, Smith BG (2000) Cell wall compositions of raw and cooked corms of taro (*Colocasia esculenta*). *J Sci Food Agric* 81:311–318
- Rahman SMM, Rakshit SK (2004) Effect of endogenous and commercial enzyme on improving extraction of sweet potato starch, Paper no.047076, ASAE Annual Meeting 2004
- Sadasivam S, Manickam A (2011) Estimation of starch by Anthrone reagent. In: Biochemical methods. New Age International Publishers, New Delhi, India. 9–10
- Sandhu KS, Singh N (2007) Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chem* 101:1499–1507
- Shah N (2007) Optimization of an enzyme assisted process for juice extraction and clarification from litchis (*Litchi Chinensis* Sonn.). *Int J Food Eng* 3(3), Article 8, DOI:10.2202/1556-3758.1073
- Singhal RS, Kulkarni PR (1990) Some properties of *Amaranthus paniculatus* (Rajgeera) starch pastes. *Starch-Starke* 42:5–7
- Sugimoto Y, Ohnishi K, Takaya T, Fuwa H (1979) Comparative susceptibility to pancreatin of starch granules from different plant species. *J Jpn Soc Starch Sci* 26:182–190
- Tari AT, Singhal RS (2002) Starch based spherical aggregates: stability of a model flavoring compound, vanillin entrapped therein. *Carbohydr Polym* 50:417–421
- Torrucó-Uco J, Betancur-Ancona D (2007) Physicochemical and functional properties of makal (*Xanthosoma yucatanensis*) starch. *Food Chem* 101:1319–1326
- Wanasundara UN, Shahidi F (1998) Concentration of ω -3 polyunsaturated fatty acids of marine oils using *Candida cylindracea* lipase: optimization of reaction conditions. *J Am Oil Chem Soc* 75:1767–1774
- Wischmann B, Ahmt T, Bandsholm O, Blennow A, Young N, Jeppesen L, Thomsen L (2007) Testing properties of potato starch from different scales of isolations—a ringtest. *J Food Eng* 79:970–978
- Zhao J, Whistler RL (1994) Spherical aggregates of starch granules as flavor carriers. *Food Technol* 48:104–105