Effect of enzyme concentration, addition of water and incubation time on increase in yield of starch from potato

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Abstract Enzymatic treatment process for starch extraction from potato was investigated using cellulase enzyme and compared with conventional process. The effects of three parameters, cellulase enzyme concentration, incubation time and addition of water were evaluated for increase in starch yield as compared to the conventional process i.e., without using enzyme. A two-level full factorial design was used to study the process. The results indicated that all the main parameters and their interactions are statistically significant. Enzyme concentration and incubation time had a positive effect on the increase in starch yield while addition of water had a negative effect. The increase in starch yield ranged from 1.9% at low enzyme concentration and incubation time and high addition of water to a maximum of 70% increase from conventional process in starch yield was achieved when enzyme concentration and incubation time were high and addition of water was low suggesting water present in the ground potato meal is sufficient for access to the enzyme with in the slurry ensuring adequate contact with the substrate.

Keywords Potato · Starch · Enzyme · Extraction

Introduction

Potato (*Solanum tuberosum* L.) is a member of the Solanaceae family and is of significant importance as a source of edible starch. It is native of South America and is primarily a temperate crop. In India potato has been in cultivation since its introduction in the early part of the 17th century (Choudhury 2003). Starch is one of the most abundant carbohydrates present in nature. It is widely distributed amongst the plant kingdom from seeds and stems to leaves and roots. It is a major industrial raw material. It is used in diverse products from papers, textiles, adhesives, pharmaceuticals, prepared foods and plastics (Nand et al. 2008; Mweta et al. 2008). Cereals, roots and tubers are major sources of industrial starch production. In most parts of the world maize provides the cheapest source of starch and in India starch is mainly extracted from maize and cassava. Starch from tuber crops have specific quality characteristics which may be used to for processing of different food products which require unique properties of the processed products (Fuglie 1998). In India there is problem in storing large quantities of potato during periods of high temperatures, due to shortage of cold storage space in many states, erratic electric supply and costly cold storage facility. Properties of native starch is also altered during storage which makes them unsuitable for certain use (Ezekiel et al. 2010). Starch can be extracted from potatoes which can find specific uses in food and other industries and also alleviate the problem of storing large quantities of potato during periods of high temperature (Marwaha 1997).

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).

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The starch granules present in the roots and tubers are embedded in cellulosic fibers and held together by pectin substrates (Rahman and Rakshit 2004). In industrial processes potato starch is mainly extracted by mechanical disintegration of the cell wall and washing out of the starch granules by water (Joshi and Kulkarni 1993). Such methods are energy intensive and may result in a lower starch recovery; also the starch granules are damaged which affects the properties of the starch. Enzymes can be used to get higher recovery of starch from tuber crops. The most distinctive feature of enzymes is that they can effectively operate at mild physiological conditions, at atmospheric pressure, temperatures up to 100 °C and pH of 3–10 (Klacik 1988).

Several investigations showed that enzymes can be used to get higher recovery of starch and to reduce the protein content of extracted starch from cereals (Johnston and Singh 2004; Serna-Saldívar and Mezo-Villanueva 2003; Zheng and Bhatti 1998). It can also be used to reduce the steeping time of cereals (Johnston and Singh 2004; Steinke and Johnston 1991). Several authors have reported that recovery of starch from tubers can also be increased by using enzymes or by fermentation (Dzogbefia et al. 2008; Gayal and Hadge 2003; Padmanabhan et al. 1993; George et al. 1991).

The present study was taken up because during times of overproduction much of the potatoes go waste in spite of having cold storages. If the starch from these excess potatoes can be extracted, the starch can be used for other purposes and also storing the extracted starch is convenient. In the present study the effects of cellulase concentration, incubation time and addition of water on recovery of starch were investigated. The main aim of the work is to examine whether cellulase treatment facilitates better recovery of starch from potato tuber as compared to conventional process.

Materials and methods

Materials

Potato variety ‘Kufri Jyoti’ was used for the experiments. The variety was collected from the farm of G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India. The average starch content of the variety was found to be 13.36%. Cellulase enzyme procured from Bionic Naturals Pvt. Ltd., Delhi, India was used for all the experiments. The Filter Paper Activity of the enzyme was found to be 1300 IU/g of cellulase.

Experimental design

A three factor two-level full factorial design was used with the following levels: Enzyme concentration (EC) of 0.1 and

0.4 g/100 g potato; Addition of water (BD) of 10 and 50 ml/100 g potato; and incubation time (IP) of 1 and 6 h. The response for the treatments was increase in starch extracted (SEI) in percent (%) from the control. In the present study control treatment means starch extracted without adding enzyme and without diluting the ground potato.

Extraction of starch

Starch was extracted as per the method described by Benesi et al. 2004 with modifications. The potatoes were washed under tap water so that any dirt adhered to it may be removed. After washing the potatoes were peeled and cut into small pieces of approximate 1 cm³. 100 g of cut potatoes were weighed and again washed in water and grinded in high speed commercial blender (Philips HL1632) for 2 min. The ground potato was then transferred to a conical flask and appropriate amount of water was added for addition of water. Enzyme was added to the ground potato as per the experimental design. The flask was cotton plugged and kept for incubation in incubator-shaker at 45 °C with a shaking speed of 125 rpm. The pH of all the samples was between 6 and 7 and cellulase enzyme is effective between pH 3 and 7. So, the pH of the broth was not changed. After incubation the resultant slurry was suspended in 500 ml of water and filtered through a double fold muslin cloth into a beaker. The filtrate was allowed to settle for 1 h to separate the starch from the other components. After sedimentation the supernatant was discarded. The top brown layer of the sediment was removed carefully by scraping and the settled starch washed with water and again kept for sedimentation for 1 h. The separated starch was dried in hot air oven at 50 °C for 24 h to reduce the moisture content of the starch to 12%.

Increase in starch extracted (SEI)

In the present study the material obtained after drying is referred to as starch extracted. The starch extracted is determined as starch extracted (SE)=(weight of the material after drying×100)/(weight of ground potato).

$$\text{SEI}(\%) = (\text{SE of treatment} - \text{SE of control}) / \text{SE of control in percent}$$

The measurement of weights was done on Mettler AE 166 balance (Capacity 150 g, Least count: 0.0001 g).

Moisture content

Moisture content of the dried materials was measured by hot air oven method (Ranganna 1997).

Data analysis

All the experiments were conducted in triplicates. Analysis of variance (ANOVA) using Design Expert Software was conducted to see the significance of the main effects as well as their interactions on the response. The Least Significant Difference (LSD) method was used to determine the statistical difference between the eight treatment results for the response.

Results and discussion

Effect of main parameters and their interactions on increase in starch extracted

Enzyme concentration and incubation time greatly affected the increase in starch extracted while effect of addition of water was less as compared to the other two main parameters (Table 1). The effect of the interactions of two parameters was also found to be significant (Table 1). Statistical analysis also showed that lack of fit for a two factor interaction model was not significant which is good. From the results (Table 2) it can be seen that the increase in starch extracted was highest (70.0%) when EC and IP were high and BD was low and lowest (1.9%) when EC and IP were low and BD was high.

Comparing the results it can be seen that SEI was more EC was high and SEI was less when EC was less. The reason for this may be that as more amount of enzyme is added more cellulosic materials, present in the potato tubers, are hydrolyzed releasing more amount of starch (Serna-Saldívar and Mezo-Villanueva 2003 and Akhtar et al. 2001). Similar trend was also observed for IP. The

increase in SEI with increasing IP may be due to the enzyme getting more time to hydrolyze the substrates. But, with BD the trend is opposite. This could be because enzyme concentration available for hydrolysis may be higher in low addition of water where as the enzyme gets diluted at higher volumes (Spanheimer et al. 1972).

Effect of two factor interaction on increase in starch extracted

Figure 1 (a) represents the effect of interaction of EC and IP on SEI. It can be observed from the figure that at low level of BD the difference in SEI was less for the two levels of IP when EC was low and the difference increased when EC was increased. Similar results were observed when BD was high. Similarly when IP was high the difference in SEI was more for different levels of EC. Also, no significant difference existed between SEI for the two levels of EC when IP was low and at high BD. This may be due to less time available for the enzyme to hydrolyze the cellulose (Johnston and Singh 2004).

Figure 1 (b) illustrates the two factor interaction between EC and BD on SEI. It is clear from the figure that at high EC the SEI is greatly affected by change in BD. Also, it was observed that SEI was more when BD was less and other factors were constant. Two possibilities arise for the high at low addition of water i.e. 10 ml/100 gPM; (i) the enzyme concentration available for cellulolysis may be higher in low broth addition of water where as the enzyme gets diluted at higher broth volumes (ii) there is a chance of sedimentation of the substrate particles at higher broth volumes due to a longer settling column available and hence the contact between the suspended enzyme and sediment substrate could be reduced, leading to poor

Table 1 ANOVA for two factor interaction model for SEI

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F	Remarks
Model	11801.4	6	1966.9	256.6	< 0.0001	significant
EC	3978.4	1	3978.4	519.0	< 0.0001	significant
IP	4395.6	1	4395.6	573.4	< 0.0001	significant
BD	1581.1	1	1581.1	206.3	< 0.0001	significant
EC×IP	1074.7	1	1074.7	140.2	< 0.0001	significant
EC×BD	590.0	1	590.0	77.0	< 0.0001	significant
IP×BD	181.5	1	181.5	23.7	0.0001	
Residual	130.3	17	7.7			
Lack of Fit	5.8	1	5.8	0.7	0.4007	not significant
Pure Error	124.5	16	7.8			
Cor Total	11931.7	23				

SEI- increase in starch extracted; EC- Enzyme concentration; IP- incubation time; BD- Addition of water

Enzyme concentration (EC) of 0.1 and 0.4 g/100 g potato; Addition of water (BD) of 10 and 50 ml/100 g potato; and incubation time (IP) of 1 and 6 h. The response for the treatments was increase in starch extracted (SEI) in percent (%) from the control.

Table 2 Treatment combinations of EC, IP and BD and response (SEI)

EC (g/100 g potato)	IP (h)	BD (ml/100 g potato)	SEI (%)
0.1	1	10	12.8±3.79 e *
0.4	1	10	36.0±1.92 c
0.1	6	10	21.9±4.39 d
0.4	6	10	70.0±4.30 a
0.1	1	50	1.9±0.86 f
0.4	1	50	3.4±1.44 f
0.1	6	50	20.1±1.21 d
0.4	6	50	50.3±1.51 b

SEI- increase in starch extracted; EC- Enzyme concentration; IP- incubation time; BD- Addition of water

Response Reported as SEI±Std. Dev

* SEI values followed by same letters are not significantly different

hydrolysis of cellulose and hence reduced starch recovery (Spanheimer et al. 1972). There were no significant differ-

ences in SEI when IP and was BD low for different levels of EC and when EC was low and IP was high for different levels of BD. The reason may be at low EC and high IP all the enzyme molecules get sufficient time for contacting the substrates and also as the concentration was already low the SEI was not affected by addition of water.

From Fig. 1 (c) it can be seen that SEI was not much affected by BD at high IP and it is also not affected by IP at low level of BD when EC was low. But, significant differences in SEI existed between different levels of IP and BD when EC was high showing that IP and BD are significant only at high concentration of enzyme.

Conclusion

In the present study a maximum of 70% increase from conventional process in starch extracted was achieved when enzyme concentration and incubation time were high and addition of water was low suggesting water

Fig. 1 Effect of two factor interaction on SEI, (a) Effect of interaction of IP and EC on SEI at low and high level of BD, (b) Effect of interaction of BD and EC on SEI at low and high level of IP, (c) Effect of interaction of IP and BD on SEI at low and high level of EC. SEI- increase in starch extracted; EC- Enzyme concentration; IP- incubation time; BD- Addition of water

present in the ground potato meal is sufficient for the free movement of the enzyme with in the slurry ensuring adequate contact with the substrate, and with higher addition of water resulting in lesser enzyme substrate contact thereby reducing recovery of starch. Sufficient amount of starch can be recovered from potato by treating with cellulase and other cell wall degrading enzymes like pectinase, which degrades the cell wall components like cellulose and facilitates in better release of starch granules, thereby requiring less grinding and low energy input. This will help in reducing wastage during times of over production.

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