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Effect of cellulase treatment on extraction of starch from potato

[1,*]Nandan, S., [2]Umashankar, A. and [1]Sankar, C. D.

[1]Department of Food Processing Technology, Tezpur University, Assam, India
[2]Department of Post harvest Process and Food Engineering, G.B. Pant University of Agriculture and Technology, Uttarakhand, India

Abstract: The effects of cellulase enzyme concentration (EC), incubation period (IP) and broth dilution (BD) on recovery of starch from ground potato meal (PM) were investigated. The effect of EC is more significant than IP and BD and among the interactions IP × BD is more significant than EC × IP and EC × BD. Starch extracted (SE) increased significantly from 7.268 to 10.108 g/100 g PM when EC was increased from 0 to 0.4 g/100 g PM and when IP was increased from 1 to 4.5h the SE increased significantly from 7.912 to 9.348 g/100 g PM, but when the IP was further increased to 6h the change in SE was not significant suggesting that 4.5 h is sufficient to hydrolyze most of the cellulose. The SE decreased when the ground potato meal was diluted by 20 ml water /100 g PM as compared to when it was diluted by 10ml water /100 g PM. Also, the difference in SE was not significant for undiluted sample and sample diluted with 10ml water /100 g PM suggesting 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. The study showed that enzymatic hydrolysis of cell wall components like cellulose can help in better release of starch granules.

Keywords: potato, starch, cellulase, extraction

Introduction

Starch can be extracted from the potatoes and can be stored for a longer time. Starch may be used for a variety of purposes (Marwaha, 1997). It is used in the manufacture of paper and paper products, in the preparation of soups, sauces, meat products, puddings, cake, etc. Starch is also used in production of candy, ice cream, and some of our most popular beverages, both alcoholic and non alcoholic.

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).

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).

An overall enzymatic milling process for extraction of starch from corn was optimized by Johnston and Singh (2004). After soaking and first grind optimization, enzyme concentration and pH determinations were evaluated using bromelain (protease obtained from pineapple plant). Results showed that the minimum addition of bromelain to reach starch yields equivalent to conventional yields were 0.4g protein/kg of corn.

Gayal and Hadge (2003) investigated the effect of using cellulase from *Penicillium funiculosum* on isolation of starch from potato. Potato cubes were subjected to cellulase treatment. Hydrolysis of cellulose and release of starch were assessed at various enzyme concentrations, with different incubation periods. About 68% starch was recovered in 6 hours and the recovery was increased to 90% in 2 hours by supplementing with pectinase.

Addition of commercial enzyme increases the extraction of starch from tuber crops considerably even at lower levels of rasping (Rahman and Rakshit, 2004; Kallabinski and Balagopalan, 1994; Padmanabhan et al., 1993; George et al., 1991). Mixing and optimum temperature for action of the enzymes during incubation increases starch yields.

The present study was taken up because during times of overproduction much of the potatoes go

*Corresponding author.
Email: *nandan@tezu.ernet.in*

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 (0 to 0.4 g/100 g potato meal), incubation period (1 to 6 h) and broth dilution (0 to 50 ml/ 100 g potato meal) 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.

Extraction of starch

Starch was extracted by the method described by Kallabinski & Balagopalan, 1994, with some modifications. Enzyme solution was prepared by mixing thoroughly 1g of the enzyme in 10ml of distilled water by a glass rod in a 20ml test tube. The potatoes were washed under tap water so that any dirt adhered to it may be removed. After washing the potatoes were cut into small pieces without peeling with a stainless steel knife to facilitate grinding. Grinding was done in Commercial (Sumeet) grinder having motor rpm of 15000 for 1 min and 15 s after standardizing the time. The ground potato meal was then transferred to a 500 ml conical flask and appropriate amount of water was added to the meal. The prepared enzyme solution was added to the potato meal using a pipette. For concentration of 0.1g per 100g of potato meal, 1ml of the enzyme solution was added to 100g of potato meal. The flask was cotton plugged and kept in incubator cum shaker at 45°C with a shaking speed of 125 rpm. The pH of all the samples varied between 6 and 7 and cellulase enzyme is effective between pH 3 and 7. So, the natural pH of the broth was not changed. After incubation the resultant slurry was screened by a nylon tea strainer of mesh size of 100 into a 400 ml beaker. During screening the pomace was washed two times in 150 ml of tap water. Sedimentation was done for 1 h to separate the starch from the other components of the filtrate containing starch. As the starch granules had higher density, they settled at the bottom. The top brown layer was removed by scraping and the settled starch washed with water and again kept for sedimentation for 1 h. After sedimentation the supernatant was discarded. The separated starch was dried in hot air oven at 50°C for 24 h to reduce the moisture content of the starch to 15% (wet basis) as most commercial starches have moisture content between 15 and 18% (wet basis).

Starch extracted

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 meal (PM) taken). 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.

Experimental design

A 5 × 5 × 5 full factorial experimental design was applied with five levels each of enzyme concentration, EC (0, 0.1, 0.2, 0.3, 0.4 g/ 100 g PM), incubation period, IP (1, 2, 3, 4.5, 6 h) and broth dilution, BD (0, 10, 20, 35, 50 ml/ 100g PM). Two replications have been carried at different times for all the 125 treatments, and each time the experiments were conducted in duplicates. So, the data for each treatment is average of four samples.

Data analysis

Analysis of variance (ANOVA) was used to test the main effects of enzyme concentration (EC), incubation period (IP) and broth dilution (BD) on starch extracted (SE) from 100 g of potato meal (PM) and also their interactions. A second order equation was developed for starch extracted and based on the equation iso-recovery curves were plotted to analyze the effect of interactions on SE. Softwares used were Minitab 13 and Surfer 7.

Results and Discussions

Overall effect of independent parameters on extraction of starch

Statistical analysis (Table 1) showed that the independent parameters (IP, EC and BD) and their interactions (EC×IP, EC×BD, IP×BD and EC×IP×BD) were found to affect the starch extracted significantly

Table 1. ANOVA for starch extracted

Source	DF	SS	MS	F
EC	4	125.684	31.4209	312.86**
IP	4	48.174	12.0435	119.92**
BD	4	49.717	12.4293	123.76**
EC × IP	16	13.466	0.8416	8.38**
EC × BD	16	7.463	0.4664	4.64**
IP × BD	16	20.904	1.3065	13.01**
ERROR	64	6.428	0.1004	
TOTAL	124	271.836	2.1922	

** Parameter significant at 1% level

Table 2. Coefficients of the equation for Starch Extracted

Predictor	Coefficient	St. dev	t-ratio
Constant	7.2225	0.3433	21.04***
EC	5.718	1.610	3.55***
IP	0.3310	0.1630	2.03**
BD	-0.02975	0.01320	-2.25**
EC^2	-1.457	3.249	-0.45 (ns)
IP^2	-0.04657	0.02115	-2.20**
BD^2	-0.000071	0.0002115	-0.34 (ns)
EC × IP	1.1054	0.2162	5.11***
EC × BD	-0.07420	0.02162	-3.43***
IP × BD	0.004905	0.001720	5.85***

*** Significant at 1% level
** Significant at 5% level
ns Not significant

Table 3. Average starch extracted for different levels of enzyme concentration, incubation period and broth dilution.

Enzyme Concentration, g/100g PM	Starch Extracted, g/100g PM	Incubation Period, h	Starch Extracted, g/100g PM	Broth Dilution, ml/100g PM	Starch Extracted, g/100g PM
0	7.268^{a*}	1	7.912^{a}	0	9.288^{a}
0.1	8.112^{b}	2	8.144^{b}	10	9.296^{a}
0.2	8.668^{c}	3	8.804^{c}	20	9.152^{a}
0.3	9.508^{d}	4.5	9.348^{d}	35	7.912^{b}
0.4	10.108^{e}	6	9.456^{d}	50	8.016^{b}

* Values followed by same superscripts in the same column are not significantly different ($p < 0.01$)

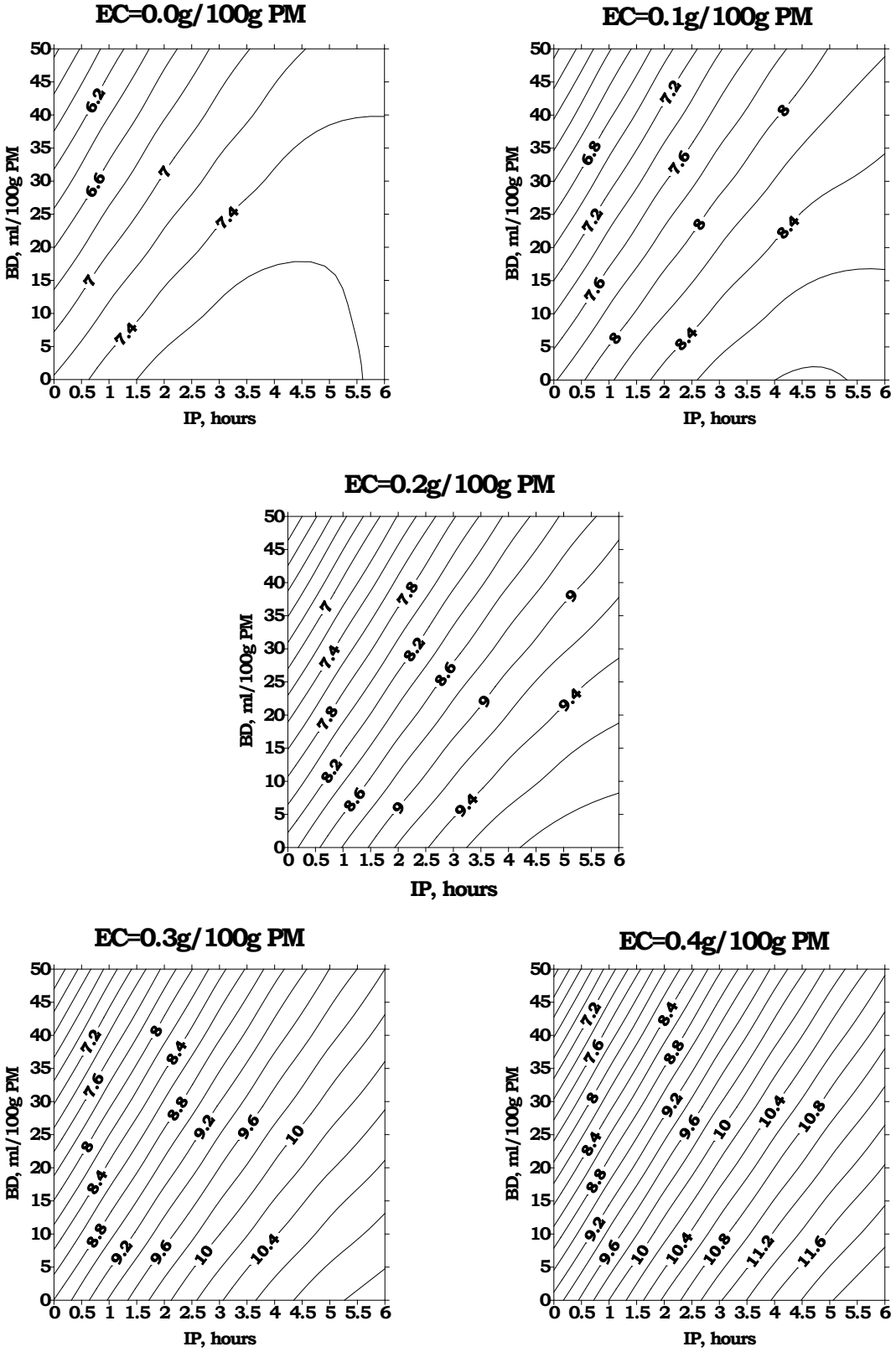

Figure 1. Effect of interaction of BD × IP on SE for different levels of EC

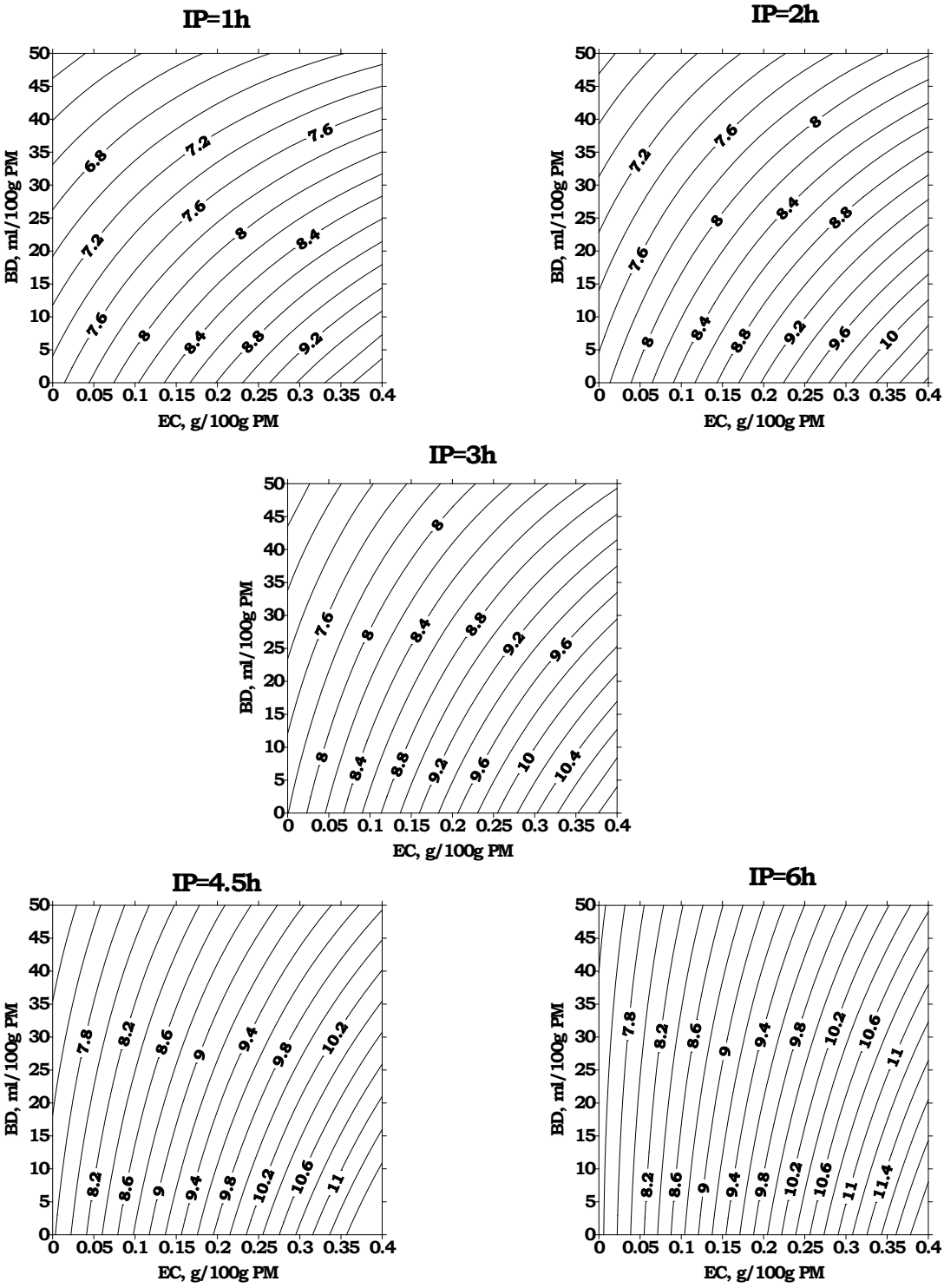

Figure 2. Effect of interaction of BD × EC on SE for different levels of IP

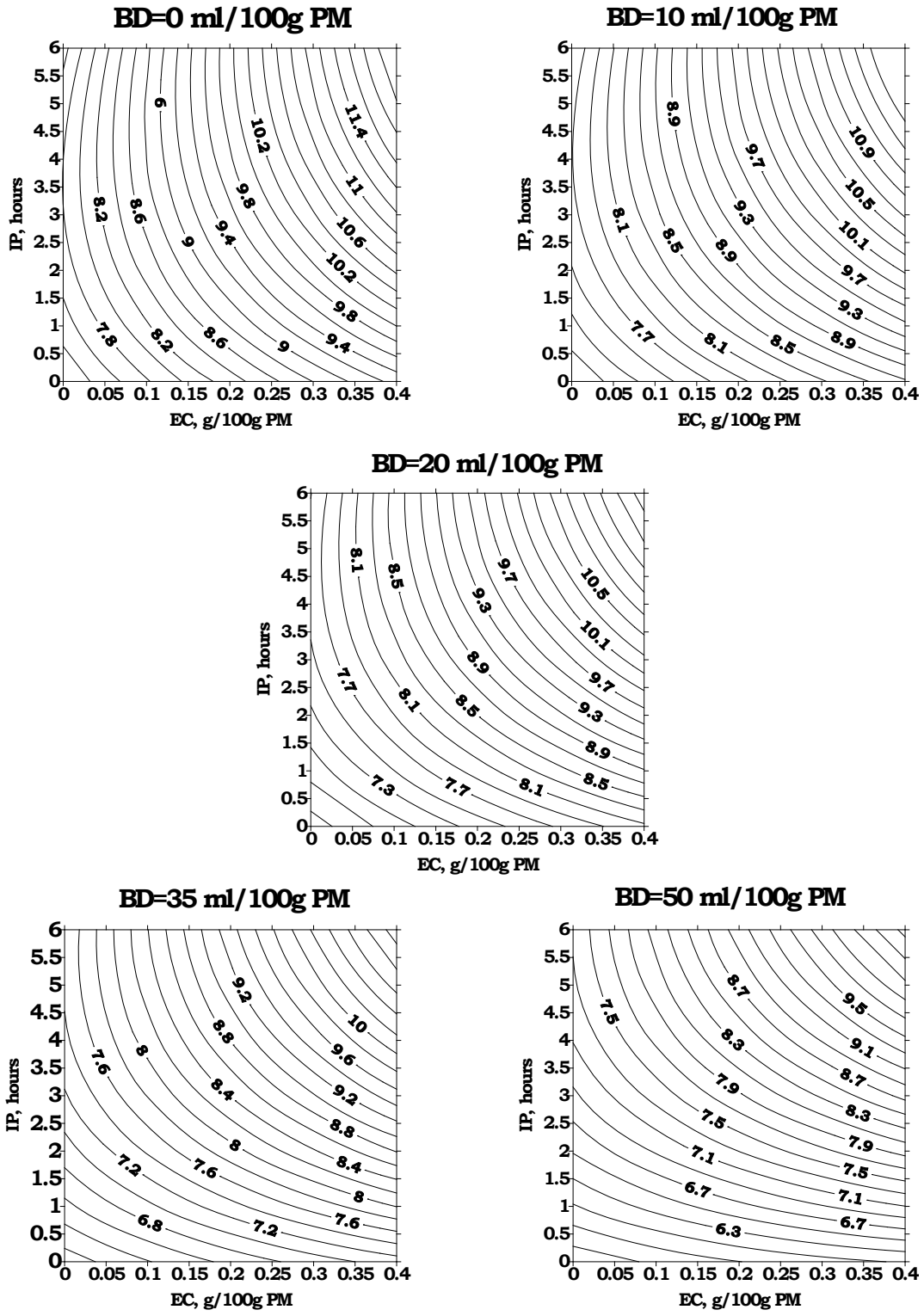

Figure 3. Effect of interaction of IP × EC on SE for different levels of BD

($p<0.01$). Further comparing the F- values it was found that the effect of enzyme concentration on starch extracted was most significant. Among the interactions the effect of IP × BD is more significant than the other two interactions i.e., EC × IP and EC × BD.

As all the parameters and their interactions were found significant, a second order regression equation ($R^2 = 84.4\%$) was developed.

The Coefficients for the equation, their t-ratio and significance is given in Table 2.

The equation is as follows

$$SE = 7.22 + 5.72\ EC + 0.331\ IP - 0.0298\ BD - 1.46\ EC^2 - 0.0466\ IP^2 - 0.000071\ BD^2 + 1.11\ EC \times IP - 0.0742\ EC \times BD + 0.0049\ IP \times BD$$

Iso-recovery curves were also developed with the help of the equation to elucidate the interactive effects of EC×IP, EC×BD and IP×BD (Figures 1, 2 and 3). The starch extracted increased when EC increased from 0 to 0.4 g/ 100 g PM and when IP increased from 1 to 6 h, but with BD the trend was not such i.e. starch extracted did not increase with increase in broth dilution. When BD was increased from 0 to 10 ml/100 g PM, SE increased and when BD was further increased SE decreased.

Effect of enzyme concentration on starch extracted

Starch extracted increased with increase in enzyme concentration (Table 3). Average starch extracted (data averaged for all values of IP and BD for each level of EC) increased from 7.268 to 10.108 g/ 100 g PM when enzyme concentration increased from 0 to 0.4 g / 100 g PM. There was significant increase in SE for any two consecutive levels of EC. 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; Akhtar et al., 2001)

Effect of incubation period on starch extracted

Average starch extracted (data averaged for all values of EC and BD for each level of IP) increased with increase in incubation period (Table 3). When the incubation period was increased from 1 to 4.5 h starch extracted significantly increased from 7.912 to 9.348 g/100 g PM. When the IP was further increased from 4.5 to 6 h, increase in the starch extracted was not significant ($p < 0.01$). This means that the increase in SE with IP was at a diminishing rate beyond 4.5 h of incubation period. The reason for such behavior may be due to the fact that by 4.5 h most of the cellulosic materials present are hydrolyzed to glucose and the remaining amount of starch granules not released by grinding and embedded in the cellulosic fibres are released. Another possible reason may be that after 4.5 h much product has accumulated which inhibits the enzyme from acting on the substrate thereby allowing release of less amount of starch.

Effect of broth dilution on starch extracted

Starch extraction was maximum at 9.296 g/100 g PM when broth dilution was 10 ml/100 g PM and minimum at 7.912 g/100 g PM when dilution was at a rate of 35 ml/100 g of potato meal (Table 3). Statistically, when the dilution with water was increased from 20 to 35 ml water/100 g PM there was significant decrease from 9.296 to 7.912 g/100 g PM in the extracted starch. But, from 0 to 20 ml the effect was not significant, and also when it is increased from 35 to 50 ml the difference was not significant.

Two possibilities arise for the increased recovery of starch at low broth dilution eg. 10 ml/100g PM; ie. (i) the enzyme concentration available for cellulolysis may be higher in low broth dilution 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 sedimented substrate could be reduced, leading to poor hydrolysis of cellulose and hence reduced starch recovery (Spanheimer et al., 1972).

Combined effect of incubation period and broth dilution on starch extracted at different levels of enzyme concentration

It is well known fact that when incubation period or enzyme concentration or broth dilution increases more of substrate is converted to product by enzymes. But, their combined effect is not much studied. So, iso-recovery curves were plotted to study the effects of interaction of two parameters keeping the third one constant. Iso-recovery curves for starch in BD-IP domain for different levels of EC were plotted (Figure 1) to study the interactive effect of IP and BD on SE.

Comparison of these graphs shows that for all enzyme concentrations, starch recovery was minimum in upper left corner (i.e., when BD is maximum and IP is minimum) and maximum in lower right zone (i.e., at lower BD and higher IP). Iso-recovery curves, for minimum recovery, increased from 5.6 to 6.2 g/100 g PM, while those for maximum recovery

increases from 7.6 to 12.0 g/100 g potato meal (with the increase in enzyme concentration from 0.0 to 0.4 g/100 g PM). The increase in recovery was diagonal (perpendicular to iso-recovery curves) such that it increased with increase in IP and decrease in BD. the iso-recovery curves are more closely spaced for higher values of enzyme concentration, than those for lower concentration. This indicates that rate of change of starch recovery with IP and BD was greater at higher enzyme concentration. Number of iso-recovery curves was slightly more for change in IP at a particular BD than that for change in BD at a particular IP. This means that incubation period has a slight greater effect on starch recovery as compared to broth dilution. Number of iso-recovery curves for BD was more when IP was low and decreased when IP was increased. Similarly, number of iso-recovery curves for IP was more when BD was high and decreased with increase in BD. This means that effect of BD was more prominent for lower incubation period and effect of IP was more prominent at higher broth dilution.

The reason for decrease in SE, as also observed by Spanheimer et al. (1972), when BD is increased may be that when the dilution was more some of the enzyme present in the solution may never have come into contact with the cellulose, thereby unable to react with the substrate. Increase in IP increased SE at particular EC because the enzyme gets more reaction time, so more cellulose present in the cell walls of potato tuber was hydrolyzed and the intracellular starch was released. When EC was more the effect of BD and IP becomes more prominent because more enzymes are present, therefore the reaction rate increases and more cellulose present in the tubers were hydrolyzed.

Combined effect of enzyme concentration and broth dilution on starch extracted at different levels of incubation period

Comparing the iso-recovery curves in IP-EC domain (Figure 2) it can be seen that for all incubation periods, the starch recovery was minimum in upper left corner (i.e., when BD was maximum and EC was minimum) and maximum in the right lower corner (i.e., when BD was minimum and EC was maximum). The value of iso-recovery curves, for minimum recovery increased from 6.2 to 7.4 g/100 g PM and those for maximum increased from 9.8 to 12 g/100 g PM (with the increase in incubation period from 1 to 6 h). The gap between two consecutive iso-recovery curves decreased as the IP was increased from 1 to 6 h. This indicates that rate of change of starch recovery with EC and BD was greater at higher incubation period. When the incubation period was increased from 1 to 6 h the nature of the iso-recovery curves changes. Initially, when IP was low, the increase in starch recovery was diagonal, but as the IP was increased the curves become almost parallel to the BD-axis. This means that when IP was more the effect of BD decreases. The reason for this may be that when IP was more the enzyme which was not able to come in contact with the cellulose when BD was more, might have come in contact with substrate, thereby releasing more starch and decreasing the effect of broth dilution. The curves are thinner in the upper left portion of the EC-BD domain. This means when EC was low and BD was high the rate of change in starch recovery was low.

Combined effect of enzyme concentration and incubation period on starch extracted at different levels of broth dilution

Iso-recovery curves for starch in IP-EC domain (Figure 3) shows that for all levels of broth dilution the starch recovery was minimum in the lower left corner (i.e., when enzyme concentration and incubation period both were minimum) and maximum in the upper right corner (i.e., when enzyme concentration and incubation period were maximum). Iso-recovery curves, for minimum recovery, decreased from 7.4 to 5.7 g/100 g PM, while those for maximum recovery decreased from 12.0 to 10.5 g/100 g PM (with the increase in broth dilution from 10 to 50 ml/100 g PM). Number of iso-recovery curves for IP was less when EC is low and more when EC was high. Similarly, number of iso-recovery curves for EC was less when IP was low and more when IP was high. This indicates that IP and EC affect the recovery to a lesser extent when EC and IP were low respectively. The iso-recovery curves were more closely packed at the upper right region indicating that the rate of recovery of starch was greater when IP and EC were higher. Initially, when BD was less at lower concentrations of EC the curves were almost parallel to the IP-axis, but as the BD was increased, at low values of IP the curves were almost parallel to the EC-axis. This indicates that at lower values of BD the effect of incubation period was less when enzyme concentration was less and when BD was high the effect of enzyme concentration was less at lower values of incubation period.

Conclusion

The study suggested that higher recovery of starch can be achieved from potato by treating the ground meal with cellulase enzyme. Enzyme concentration,

incubation period and broth dilution all had significant effect on starch recovery. Starch recovery increased with increase in enzyme concentration, giving maximum recovery of 10.108 g/ 100 g potato meal at enzyme concentration of 0.4 g/ 100 g PM. Starch recovery also increased with incubation period, but after 4.5 hours of incubation the increase in the recovery was not significant. Broth dilution above 10 ml / 100 g potato meal resulted in decrease in recovery of starch, suggesting that water present in the ground potato meal was sufficient to dissolve the enzyme, and with higher dilution 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, which degrades the cell wall components like cellulose and facilitates in better release of starch granules, thereby requiring less grinding and low energy input.

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