*Polish Journal of Chemical Technology, 22, **3**, 48—54, 10.2478/pjct-2020-0027*

Enzymatic extraction of potato starch: A parametric optimization study using response surface methodology

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Optimized, effective and efficient methodology has been determined in this research work for the recovery of starch from potatoes. Potato starch extraction experimental results have been utilized for the parametric optimization study by using different statistical techniques. In this research work, starch extraction was conducted by employing cellulase enzyme. Response surface methodology (RSM) was put to use to perform statistical analysis to get optimum results. Five-level central composite design (CCD) consisting of three parameters was implemented to investigate the effect of enzyme concentration, contact time and broth dilution. Experiment results revealed that increment in enzyme concentration and contact time enhanced the starch recovery while dilution showed the inverse relation on the recovery of starch. Optimum starch recovery was achieved upto 89% when enzyme concentration (0.5 g/100 g) of potato meal was diluted with 10 mL of water and mixed for 4 h at 45°C.

Keywords: Response Surface methodology (RSM), Potato, Starc, Optimisation, Extraction Parameters.

INTRODUCTION

Starch is an essential raw material for several manufacturing processes in pharmaceutical, textile, food, adhesive, construction, paper and package industries. In 2008, total global starch utilization was 66 million ton and is projected to reach 161 million metric ton in 2024 with an annual growth rate of 4%. Starch can be extracted from more than 50 different plants and potato is the second larger source of the starch after maize. EU is the largest producer of potato starch with approximately a 14% market share globally. Potato is the third most produced and consumed crop after wheat and rice around the globe and is a source of macro and micro nutrients such as carbohydrates, proteins dietary fibers, minerals and vitamins[1, 2].

Tradionally starch has its usage in food and realted industries. However technological advancment has turn its application in many other sectors like textile, paper and fine chemicals. In addition to its ability as thickening agent, it can also be employed as an excipient and as disintigrant in pharma industry and as a source of biofuel by fermentation[3]. Despite these applications of starch, the commercial benefits are limited owing to the low yield and high cost of industrial production[4].

Different varieties of fresh potatoes consist of 75% water, 20 to 22% starch, 1% fiber and 1 to 1.5% of protein contents[5]. Potato starch is preferred choice over other starches in food industry due to its lowest content of fats, lipids and proteins[6]. It does not produce color, smell or taste during processing specially in textile, paper and pharmaceutical industry. Other advantages include better adhesion, good remoisten ability, high paste consistency, low gelatinization temperature and fine quality than that of cereal starch[7]. Its gelatinization point, amylose content and functional properties can be preserved by treating it before storage. It has been appeared in related studies that percentage of starch contents in potato crops is dependent on many factors including weather climate, soil type, fertilizer, crop variety and growth period[8, 9]. These parameters have produced account able effect on the morphological, thermal and rheological properties of the starch particles and on its extraction yield[10].

Different methods are being utilized for extraction of starch from potatoes i.e. mechanical, alkaline and enzymatic extraction[11]. Mechanical separation methodology is widely utilized in various manufacturing units around the globe for extraction of potato starch as well as cereal crops. In this methodology starch washing is mandatory with water. It helps to remove impurities and optimize starch recovery, but it is least preferred due to high energy cost[12]. Alkaline treatment is also employed for starch separation from potato, rice and sago. Alkaline treatment alters the physiochemical properties of starch particle. It minimizes the amylase percentage, enhances the swelling ability of potato and reduces the viscosity of starch particles[13]. Enzymatic treatment appeared as energy efficient approach for the extraction of starch from starch crops[14]. Enzymatic method needs a lesser amount of grinding and slighter energy inputs for starch extraction in comparison to mechanical and alkaline treatment. In this regard, various enzymes have been utilized for starch processing like heat stable α-amylase, β-amylase, protease and amylo glucosidase[15, 16].

In this work, role of cellulase enzymes have been exploited to extract better quality starch using response surface methodology (RSM) for the parametric optimization to enhance the extraction of potato starch. RSM is a statistical tool which can be employed for the optimization and improvement of various complex processes where multiple factor interfere the production yield[17, 18]. It involves the modelling of response variables based on experimental outcomes[19, 20]. RSM has already been established to collect the data for designed optimization and resulted in cutting the cost and noise, linked with associated extraction methods due to its short cycle, precise regression equation and with less number of experimental runs[18, 21].

Many methods have been used to extract starch using enzymes from rice, potato and related cereals but no investigation has been made to detrmine the exact value of enzyme concentration, contact time and broth dilution on the extraction of starch using response surface methadolgy. Thus the objective of present study was to investigate the effect of cellulase enzyme on the recovery of starch and to determine the effect of parameters such as enzyme concentration, contact time and broth dilution with water. Thus, Design Expert Software version 7.0 was employed to make design of experiments (DOE) and to draw and explore contours on interactive 2D graphs to visualize the response surface from all angles with rotatable 3D plots by putting the designed data for experiments. Therefore, five level central composite design (CCD) based on three parameters i.e. enzyme concentration, broth dilution with water and contact time of enzyme with potato for optimum extraction of starch was considered to design the experiments to determine the best suited conditions for the starch extraction.

EXPERIMENTAL

Material and methods:

Material

Potatoes were obtained from Ayub Agricultural Research Institute Faisalabad, Pakistan. Cellulase enzyme was arranged from Bio-Enzyme lab, University of Agriculture (UAF), Faisalabad, Pakistan. Other chemicals of ACS grade were purchased from Merch and used as recieved.

Equipment required

Equipment required for the solid liquid extraction of potato was an isothermal batch extraction unit fitted with thermostat, stirrer, rpm controller assembly. Other apparatus involves multitasker, grinder, conical flask, beaker, digital weight balance and nylon strain (100 mesh size) and Anton Paar Digital Polarimeter MCP-100.

Extraction procedure

Starch was recovered from potatoes by adopting the following steps. It involved cutting & grinding of raw material, addition of cellulase enzyme and distilled water, mixing of solution, heating, filtration and drying. Process flow diagram has been shown in in Figure 1. As a first step, fresh potatoes were washed and dried at room temperature for two days. Afterwards, it was weighed and cut into transverse directions followed by grinding in grinder at 1500 RPM for 2 minute to get uniform potato meal. Potato meal was transferred to 2250 mL conical flask and appropriate amount of water was added as required for each experiment. Later 1 gram of enzyme was mixed in 10 mL of water by glass rod. For a concentration of 0.1 g/ 100 g of potato meal, the 1 mL of enzyme solution was mixed in 100 g of potato meal. The flask was closed by cotton pad to stop the vapours exhaust and placed in shaker at 150 RPM for required period of contact time as per requirement of designed experiment for uniform mixing of enzyme with potato meal. pH of the solution was maintained at 5 and was adjusting with NaOH and HCl. All the experiments were performed with desired level of enzyme concentration in g/ 100 g of potato meal ranging from (0.1–0.5 g/100 g of potato meal with increment of 0.1 g), contact time period in hours ranging from (1–5 h with increment of 1 h) and broth dilution in mL ranging from (0–40 mL distilled water with increment of 10 mL). After required period of contact time, solution was passed through nylon strainer of 100 mesh size in a 500 mL beaker, collected pomace at top of screen was washed twice with distilled water for maximum recovery of starch. The filtered starch was allowed to sediment for 6 h and upper layer of the water was decanted and wet starch was dried in microwave oven to reduce its moisture content ≤5%. Finally starch sample was dried at 65°C for 24 h in hot air drying oven and then kept in air tight vial before further analysis. Later the total starch % age was determined by ISO 10520:1997 method[22]. In the course of this investigation, twenty experimental runs were designed and performed for the removal of starch from potato using cellulose enzyme along with single control. For optimization, the effect of parameters, like enzyme concentration, contact time and dilution with distilled water at various levels are represented in Table 1. All the experiments were carried out in triplicate and average value has been mentioned. The percentage removal of starch from potato was found by utilising equation (1).

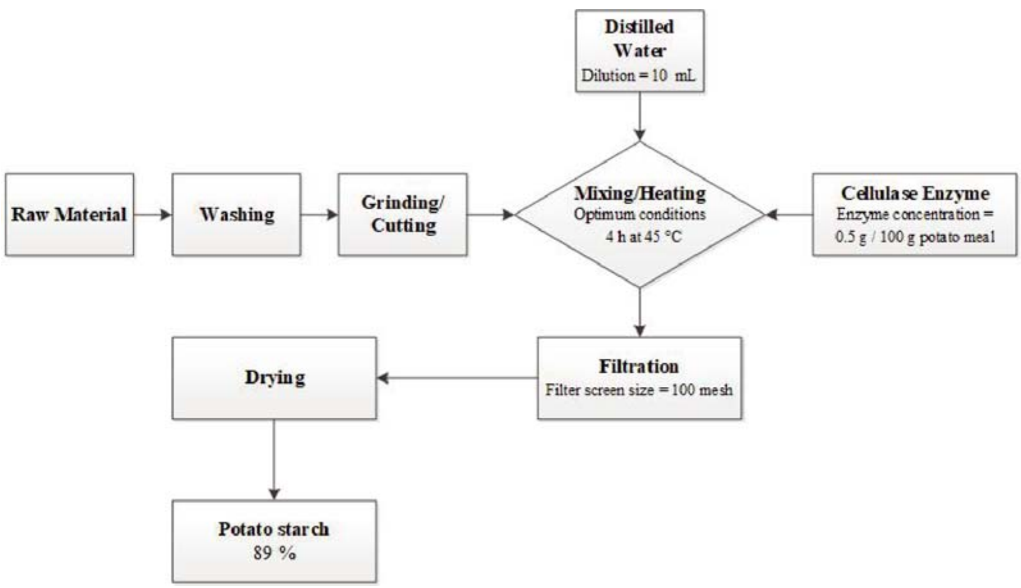

Figure 1. The schematic diagram of the experimental procedure

$$R = m_e / m_s \times 100 \quad (1)$$

Where:

R = Starch recovery percentage, %

m_e = Mass of starch removed, g

m_s = Total mass of starch in potato sample, g.

Table 1. Un-coded and coded levels of independent variable

Symbols	Variables	Units	Levels –α	–1	0	1	α
A	Enzyme Concentration	g/g	0.1	0.2	0.3	0.4	0.5
B	Dilution	mL	0	10	20	30	40
C	Contact Time	h	1	2	3	4	5

Experimental Design:

Response surface methodology (RSM) under five level CCD were employed to investigate optimum parametric values for maximum starch extraction from potatoes. Optimization was carried out for the three parameters i.e. enzyme concentration, dilution and contact time and is symbolised as follows. The mathematical expression for the recovery of starch extraction from potato is represented in equation (2). The representation of independent variables of coded and un-coded levels are shown in Table 1 and can be correlated by equation (3).

A = Enzyme concentration, g/g

B = Dilution, mL

C = Contact time, h.

$$R = f\,(A, B, C) \quad (2)$$

where:

R = Starch extracted or response

f = Response function,

A, B and C = factors, parameters or variables.

$$X = xi - xo / \Delta x \quad (3)$$

where:

X = coded value,

xi = actual value,

xo = actual variable midpoint value,

Δx = original range interval.

The values of twenty experimental runs were obtained from CCD with eight factorial points, six axial and six replicates of central point. The CCD experimental design layout matrix with their values of predicted and actual responses are shown in Table 2. It can be concluded from Table 2 that the starch recovery was the highest (*i.e.* 89.04%) at experiment 12 and lowest (*i.e.* 33.83%) at experiment 05, respectively. It was further observed that there is a close agreement with predicted and experimental values for the mentioned experiments. The experimental results were analysed using RSM to fit a second order polynomial equation (4).

$$r = \beta_o \pm \sum_{i=1}^{n} \beta\ Xi \pm \sum_{i=1}^{n} \beta\ iiXi^2 \pm \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta\ ijXiXj \quad (4)$$

where:

r = The response,

βo = Constant term,

βii = Regression coefficient of quadratic terms,

βij = Regression coefficient of interaction terms,

Xi, Xj = Coded variables,

βi = Regression coefficient of the linear terms,

n = Number of independent variables.

RESULTS AND DISCUSSION

Summary Statistics & Model Fitting

As discussed previously, three extraction parameters i.e. enzyme concentration, dilution and contact time were analysed. In Table 3 the statistical analysis of potato starch extraction using CCD is described. From these results and statistics, the highest order polynomial model was selected where model is not alias.

Table 2. CCD layout

Run	Coded values: Enzyme Concentration, g/g	Coded values: Dilution / mL	Coded values: Contact Time / h	Actual (Uncoded) values: Enzyme Concentration, g/g	Actual (Uncoded) values: Dilution / mL	Actual (Uncoded) values: Contact Time / h	*R* %: Experimental value (a)	*R* %: Predicted value
1	0	0	1	0.3	20	5	59.39	58.87
2	–1	1	1	0.1	40	5	46.29	47.07
3	0	0	0	0.3	20	3	56.82	52.82
4	0	0	0	0.3	20	3	56.05	52.82
5	–1	1	–1	0.1	40	1	33.83	34.98
6	–1	0	0	0.1	20	3	44.97	45.57
7	1	1	1	0.5	40	5	58.82	61.57
8	–1	–1	1	0.1	0	5	56.34	56.17
9	0	–1	0	0.3	0	3	51.02	57.38
10	0	0	0	0.3	20	3	55.67	52.82
11	1	–1	–1	0.5	0	1	64.99	58.58
12	1	–1	1	0.5	10	4	89.04	89.87
13	0	0	0	0.3	20	3	55.73	52.82
14	0	0	–1	0.3	20	1	43.91	46.78
15	–1	–1	–1	0.1	0	1	40.00	44.08
16	1	1	–1	0.5	40	1	41.29	49.48
17	0	1	0	0.3	40	3	50.22	48.27
18	0	0	0	0.3	20	3	56.11	52.82
19	1	0	0	0.5	20	3	65.24	60.08
20	0	0	0	0.3	20	3	56.18	52.82

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

Table 3. Statistical summary of CCD

Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	*df*	Mean Square	*F*-value	*P*-value	Source
Mean vs Total	1375.68	1	1375.68			
Linear vs Mean	27.07	3	9.02	17.81	<0.0001	Suggested
2 *FI* vs Linear	1.65	3	0.5511	1.11	0.3803	
Quadratic vs 2*FI*	2.76	3	0.9192	2.49	0.1201	
Cubic vs Quadratic	3.35	4	0.8381	14.71	0.0029	Aliased
Residual	0.3419	6	0.057			
Total	1410.85	20	70.54			
Lack of Fit Tests						
Source	Sum of Squares	*df*	Mean Square	*F*-value	*P*-value	
Linear	8.08	11	0.7349	175.31	<0.0001	Suggested
2 *FI*	6.43	8	0.8039	191.75	<0.0001	
Quadratic	3.67	5	0.7346	175.24	<0.0001	
Cubic	0.3209	1	0.3209	76.55	0.0003	Aliased
Pure Error	0.021	5	0.0042			
Model Summary Statistics						
Source	Std. Dev.	*R*-squared	Adjusted *R*-squared	Predicted *R*-squared	PRESS	
Linear	0.7117	0.7696	0.7264	0.5668	15.24	Suggested
2 *FI*	0.7045	0.8166	0.7319	–0.2125	42.65	
Quadratic	0.6078	0.895	0.8005	–0.4895	52.39	
Cubic	0.2387	0.9903	0.9692	–10.2099	394.31	Aliased

Insignificant lack of fit was achieved by focusing on maximizing the adjusted *R*-squared and predicted *R*-squared values. The linear model was suggested for the starch extraction. The inferred linear model in terms of coded variables are as follows in equation (5):

$$R = 8.29 + 1.14A - 0.7146B + 0.9489C \quad (5)$$

Model of variables which are un-coded or actual is given by the equation (6):

$$R = 5.87725 + 5.69200 \text{ Enzyme Concentration} - 0.035730 \text{ Dilution} + 0.474450 \text{ Time.} \quad (6)$$

Analysis of Variance (ANOVA)

To investigate the percentage starch extraction, analysis of variance (ANOVA) has been used to rationalize the adequacy of response model. In this regard, Design Expert Software version 7.0 was employed to make the linear model. The model successfully correlated our three independent variables to influence the starch extraction. The ANOVA is summarized in Table 4 and showed the effect of linear, quadratic and interaction terms on the predicted responses. In order to exclude the non-significant terms, step wise backward elimination model was performed. In the response, smaller *p* values accounts for the effective parameter while fitness of the model to system was expressed by the higher value of correlation coefficient (*R*-squared). From Table 4, it can be seen that *p* values found to be less than 0.05, signifying that parameters of model are effective however correlation coefficient R^2 value was 76.96%. The model *F*-value of 17.81 infers that the model is significant. For the current linear model, the values of probability *F* are less than 0.05 for factors *A*, *B* and *C* that indicates the model terms are significant. It is also worth noting that all the factor *A*, *B* and *C* affect the response. It has been further observed that lack of fit was unsubstantial however the anticipated *R*-squared was in reasonable agreement with adjusted *R*-squared, Table 4.

Model Diagnostic Plots

In RSM, individual and cumulative effect of parameters can be seen to check the responses. These graphical representations for models are plotted as a function of two variables, while keeping other variables at central level. Residuals normal probability plot is presented in graph in Figure 2a. It is inferred from normal probability plot that data is considered as normal if distributed in straight line while abnormality or error in data is represented if data is dispersed as non-linear[23]. It can be inferred from Figure 2a that the data is close to central line which clued us that the model is normal. The linear regression model is used to calculate the projected values of the response. In Figure 2b, the graph is drawn between the predicted and the actual values and it exhibits a linear relation among the predicted and actual values and depicts the fitness of the anticipated model.

Response Surface Plots

To analysis the interaction effects of the process parameters, contour plots and 3D response surface plots are made and shown from Figure 3 to Figure 5. The variation in response with respect to change in two process variable has been shown with the help of contour plot[21]. As in a model there was one response with reference to two factors thus various contour and 3D plots were generated. In a sequence, Figure 3a represents the 3D

Table 4. Analysis of variance(ANOVA) for regression model

Source	**Sum of squares**	***Df***	**Mean square**	***F*-value**	***p*-value**	**Source**
Model	27.07	3	9.02	17.81	< 0.0001	significant
Enzyme Concentration	12.96	1	12.96	25.58	0.0001	
Dilution	5.11	1	5.11	10.08	0.0059	
Contact Time	9.00	1	9.00	17.77	0.0007	
Residual	8.11	16	0.5066			
Lack of Fit	8.08	11	0.7349	175.31	< 0.0001	significant
Pure Error	0.0210	5	0.0042			
Cor Total	35.18	19				

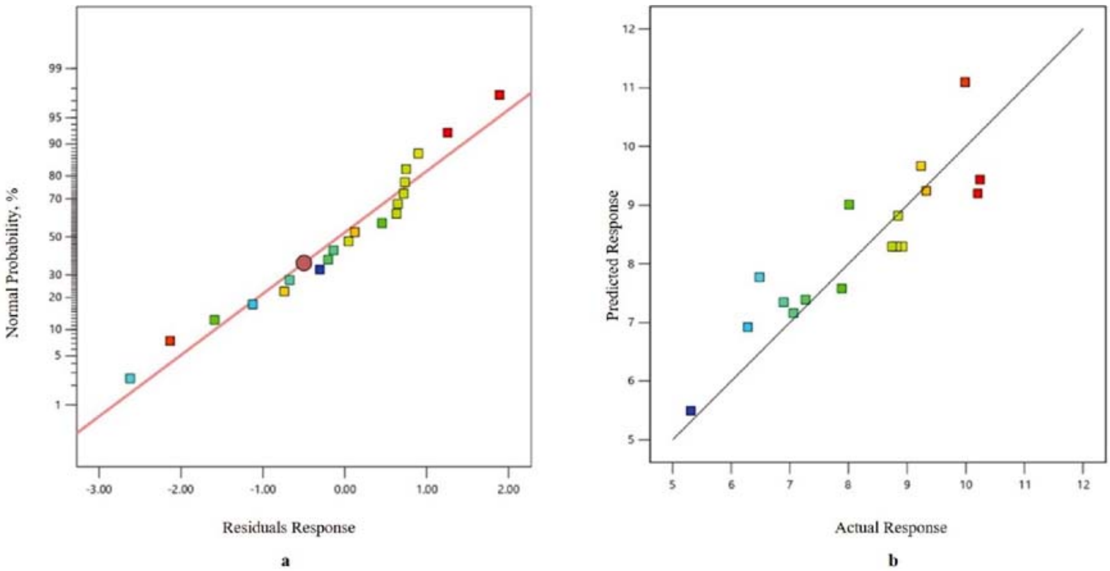

Figure 2. (a) Normal plot of residuals and (b) Plot of actual & predicted values of response

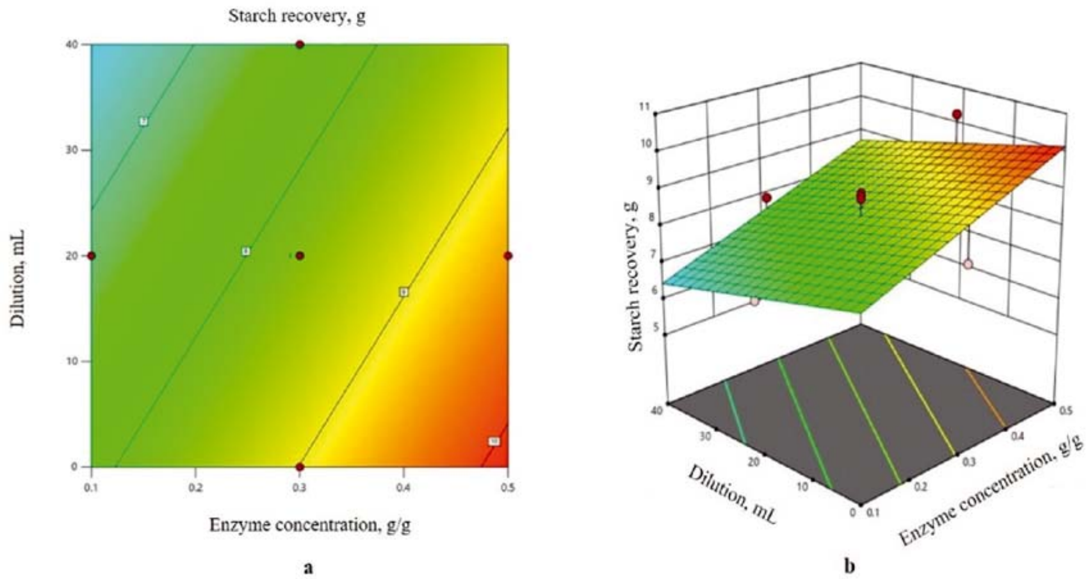

Figure 3. (a) 3d Surface plot of response for the dilution and enzyme concentration. (b) Contour plot showing relation of dilution and enzyme concentration for percentage extraction

surface plot of response and the figure 3b represents the relation of dilution and enzyme concentration for percentage extraction of starch.

The key parameter that influence the percentage starch recovery is the enzyme concentration. It can be concluded that increase in the enzyme concentration enhanced the recovery of starch. This increase in starch recovery can be attributed to greater interactions of potato in higher concentration of enzymes. Similar outcomes were observed in experimental work and cited in[24, 25, 26]. In Figure 4a and 4b change in enzyme concentration and dilution is portrayed to show the effect of percentage starch recovery. It was inferred that the percentage recovery increases by increase in enzyme concentration and decrease in dilution and thus value of response increases. The response value was found to be lower in greater dilution under the lesser dose of enzyme. Consequently, enzyme concentration at lower dilution appeared as essential factor that influence the starch recovery. It further clued us that increment of the response time with the increase in concentration of enzyme is due to the maximum contact between enzyme and potato meal at lesser dilution. Thus increase in enzyme concentration triggers the maceration or disintegration of the tissue and improves the release of starch. The outcome of enzyme concentration and contact time is shown in Figure 5a, (3D surface plot of response), and Figure 5b, (contour plot). It has been observed that by the increase in contact time, starch recovery was improved noticeably, so this is another prominent and significant parameter.

Optimization of extraction parameters

In an optimisation approach, set of process parameters has been found to furnish the maximum value of response function. In this regard, set of 20 experiments along with an additional control were designed by employing the design expert software version 7.0 to get the numerical optimisation of response variables. Outcome of the process model was successfully validated. In order to get the maximum response for the extraction of starch, design of the experiment revealed the extraction of starch within lower and upper limit of 33.83% and 89.04% respectively however control run where no enzyme was added and reaction was carried out for 4 h in

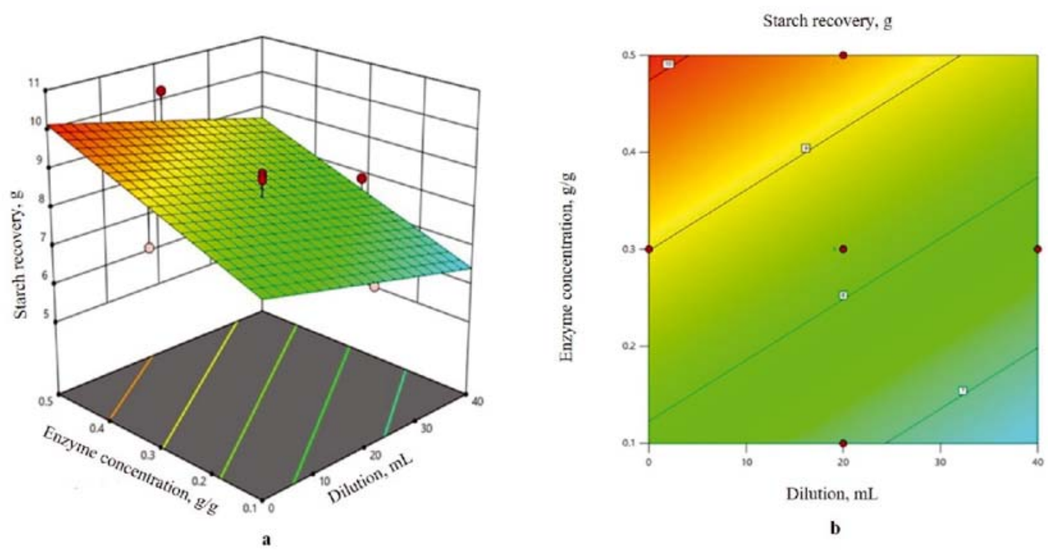

Figure 4. (a) 3d Surface plot of response for enzyme concentration and dilution. (b) Contour plot

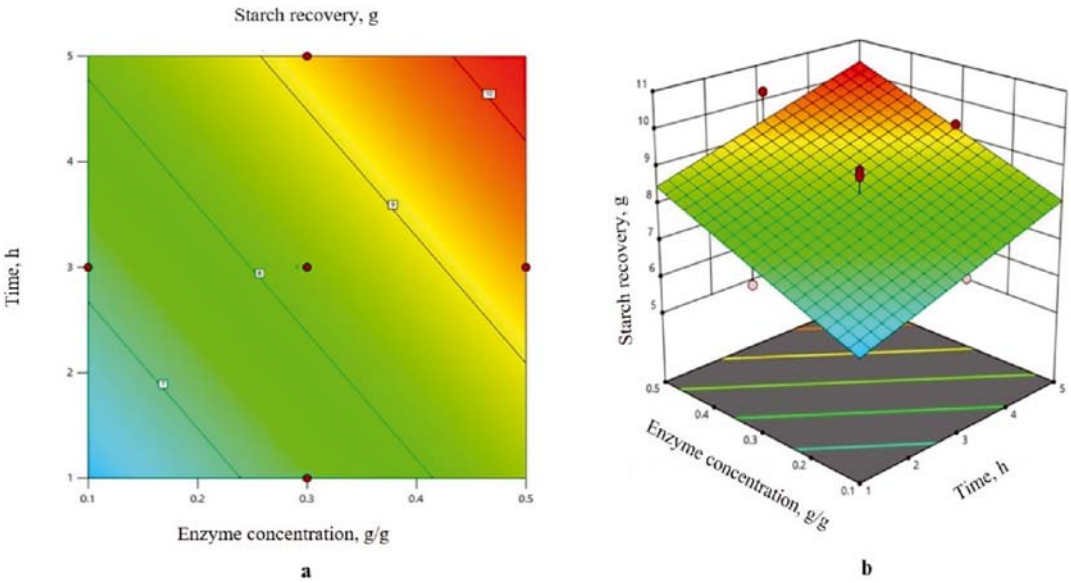

Figure 5. (a) 3d Surface plot of response with reference to enzyme concentration and contact time. (b) contour plot

10 mL provided starch in 51% yield. It indicated that all the three parameters have influential role on the % age recovery of starch and same has been depicted in the graph obtained and presented in figure 2–5. The optimal values of enzyme concentration, dilution and contact time have been found to be 0.5 g/g in 10 mL for 4 h respectively with predicted value of 89.87% at desirability of 0.969. The experiment was done on these optimum conditions and was verified.

CONCLUSION

The extraction of starch from the potato was carried out using enzymatic extraction technique. In this regard, distilled water as a solvent and cellulase enzyme was chosen for the extraction of starch from the raw chopped potato. The extraction procedure was optimised to yield the maximum recovery of starch for three parameters like enzyme concentration, broth dilution, and contact time. In this optimisation approach, RSM was employed under CCD and a linear model was developed and validated. Examination of variance (ANOVA) was done, which conclude that the main factor in this recovery was concertation of enzyme. Further, dilution of mixture with distilled water and contact time of potato with enzymes were appeared as important parameter in extraction of starch. However, time of contact was considered as another important factor and its effect was also significant. It was established that maximum yield of potato starch was found to be 89% at maximum desirability when enzyme concentration, reaction dilution and contact time was kept at 0.5 g/ 100 g (of potato meal), 10 mL and 4 h respectively.

ACKNOWLEDGEMENTS

The financial and technical support for this research is given by University of Engineering & Technology, Lahore FSD Campus Pakistan is highly appreciated.

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