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An Improved Method of Optimizing the Extraction of Polyphenol Oxidase from Potato (*Solanum tuberosum* L.) Peel

Suprabhat MUKHERJEE[1*], Bidyut BANDYOPADHAYAY[2], Bikram BASAK[3], Nilrudra MANDAL[1], Apurba DEY[3], Biswanath MONDAL[1]

[1]*CSIR-Central Mechanical Engineering Research Institute, Centre for Advanced Materials Processing, Durgapur-713209, India; suprabhat.biochem@hotmail.com (*corresponding author)*

[2]*Oriental Institute of Science and Technology, Department of Biotechnology, Burdwan, India*

[3]*National Institute of Technology, Department of Biotechnology, Durgapur, Mahatma Gandhi Avenue, Durgapur-713209, India*

Abstract

The present study has an objective to optimize the extraction of Polyphenol Oxidase (PPO) from potato (*Solanum tuberosum* L.) peel. Response surface methodology (RSM) was used to design experiments and study the effect of six influential extraction parameters: extraction buffer concentration (100-500 mM), pH of extraction buffer (4.5-8.5), time (1-12 hours), temperature (4-40°C), concentration of PMSF (1-5 mM) and volume of extraction buffer (200-1000 ml) on the extraction of PPO. The dependent variable was considered as response function which was specific activity (SA) of extracted PPO. ANOVA was performed to obtain the regression equation that could predict the responses within given range. From RSM generated model, the optimum conditions for the maximum extraction of PPO were phosphate buffer concentration of 100 mm, buffer pH of 4.5, extraction time of 1 hour, 40°C temperature, PMSF concentration of 5 mM and buffer volume of 200 ml. Finally, this study illustrates a cost effective and less time consuming method to maximize the extraction of PPO from a vegetable waste.

Keywords: ANOVA, PPO regression equation, RSM, specific activity

List of abbreviation: ANOVA: Analysis of varience; BC: Buffer concentration; C.V.: Coefficient of variation; d.f.: Degrees of freedom; EA: Enzyme Activity; ET: Extraction time; ETM: Extraction temperature; F: Fisher variance ratio; p: Probability; PB: pH of extraction buffer; PC: PMSF concentration; PPO:Polyphenol oxidase; RSM: Response Surface Methodology; PMSF: Phenyl Methyl Sulfonyl Fluoride; R:Coefficient of variation; R^2: Coefficient of determination; SA: Specific Activity; VB: Volume of extraction buffer

Introduction

PPO is a copper-containing enzyme that catalyses both molecular oxygen-dependent hydroxylation of monophenols to their corresponding o-diphenols and oxidation of o-diphenols such as L-DOPA to their cognate o-quinones (Ni Eidhin *et al*., 2010; Palma-Orozco *et al*., 2011). Important properties of PPO viz. wider substrate specificity, ability of catalyzing reaction in wider range of pH and temperature (Seo *et al*., 2003), protease activity (Gomez-Lopez, 2002) etc have been utilized for various purposes. The ability of oxidizing large group of phenolic compounds has been utilized in removal of phenolic contaminants from waste water, effluents and contaminated soil (Klibanov *et al*., 1980; López-Molina *et al*., 2003; Torres *et al*., 2003). The same property of PPO has also been utilized for removal of reactive textile dyes. Researchers have reported the use of both soluble and immobilized PPO in the biotransformation of phenolic contaminants but later provide better performance in terms of reusability and catalysis (Amjad *et al*., 2009; Duran *et al*., 2002; Niladevi and Prema, 2008). Immobilization of PPO onto porous and conducting surface has led to the development of biosensor for monitoring of aqueous phenolic components. Construction of biosensor using immobilized PPO on Carbon Nanotube (Mohammadi *et al*., 2009) and calcium carbonate nanoparticles (Shan *et al*., 2007) are the better examples of this fact. Since the enzymatic browning by PPO causes decrease of nutritional quality and affects the appearance of food, inactivation of PPO is desirable for preservation of foods (Langdon, 1987; Lee *et al*., 2007).

All these mentioned purposes require PPO either in crude or purified form. Therefore the need, demand and market value of PPO in its different fields of application is quite high. However, high production cost has limited the feasibility of its uses in industries. The cost of the sources of PPO is also a matter of concern. Keeping in view of the usefulness and cost effectiveness of PPO for various industrial purposes, it is very important to develop some analytical methods for the extraction of this enzyme in such a way that will be of high yield and cost effective. Usually, the method for determining optimal conditions in extraction processes is varying one parameter while keeping others at a constant level. This is a time consuming and cost

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ineffective method that does not include interaction effects among variables (Ranjan *et al*., 2009). Optimization employing central composite design (CCD) and response surface methodology (RSM) can overcome such drawbacks (Bas and Boyaci, 2007; Vohra and Satyanarayana, 2002). Researchers have reported the utility of CCD in optimization of experimental determinants (Ebrahimpour *et al*., 2008; Hameed *et al*., 2009) and RSM for process development as it provides all the information regarding the combinatorial effects of variables, regression modeling and optimization of the variables to maximize the desired product (Chen *et al*., 2002; Gaur *et al*., 2008; Manohar and Divakar, 2004; Sztajer *et al*., 1988).

Although considerable research works have been carried out on extraction, purification and characterization of PPO from various fruits and vegetables (Aydemir, 2010; Marri *et al*., 2003; Sener and Ümit Ünal, 2011; Yang *et al*., 2001). There was no evidence on the improvement of extraction of PPO from potato peel through statistical optimization. In this paper, RSM was conducted to study the effects of different influential extraction parameters to maximize the yield of PPO from potato peels. The optimum extraction conditions (environmental, process and solution parameters) were predicted and validated using statistical methodologies.

Materials and methods

Plant materials

Potato (*Solanum tuberosum*) peels were collected from the hotels near National Institute of Technology, Durgapur. These peels were washed several times with double distilled water and used for study. The reasons behind the selection of potato peels as experimental material were firstly, researchers have reported the presence of PPO in potato (Do-Yoon and Woo-Yean, 1996; Thygesen *et al*., 1995). Secondly, potato tuber is mainly used as the source of dietary carbohydrate whereas peels are mainly considered as waste and easily available. Therefore it is an economic source of PPO.

Chemicals

Di-sodium hydrogen phosphate (Na_2HPO_4), Sodium di-hydrogen phosphate (NaH_2PO_4), Sodium Hydroxide (NaOH) and Catechol (Pyrocatechol) were purchased from Merck (Germany). Triton X-114 (Sigma-Aldrich, USA) and PMSF (Sisco research laboratory, India) were used in this study. All the chemicals used were of analytical grade commercially available in India.

Development of suitable design matrix

RSM was employed to find the optimum experimental condition with definite values of key experimental determinants for the maximum yield of PPO in the potato peel extract. The experimental design and statistical analysis was performed using design expert software. All the experiments were designed using central composite design (CCD) with a quadratic model in order to study the combined and individual effects of six influential experimental parameters on the extraction of PPO. These variables were A: concentration of the extraction buffer (BC), B: pH of extraction Buffer (PB), C: extraction time (ET), D: extraction temperature (ETM), E: PMSF concentration in extraction buffer (PC) and F: volume of extraction buffer (VB). Each parameter had two levels which were-1 and +1, shown in Tab. 1. A total of 74 sets of experiments were performed to determine significant factors for the extraction of PPO.

Specific activity (SA) of extracted PPO was considered as the only dependent variable or response function. As the objective of this study was to optimize the yield of PPO in the extract, the response, SA has been considered as key factor because optimization of SA will ensure maximum activity of PPO per mg of protein in the extract. Relationships between six parameters (BC, PB, ET, ETM, PC and VB) and process responses (SA) for the extraction of PPO were analyzed using RSM. All the experimental conditions and value of the corresponding response studied are depicted in Tab. 2.

Extraction of PPO

One hundred g of potato peel was blended with phosphate buffer of different concentration and pH under different experimental conditions based on the combinations programmed by RSM (as per Tab. 2). 1% Triton X-114 was also added to the extraction medium. The extraction was performed under continuously stirring using magnetic stirrer. After extraction each mixture was centrifuged at 18,000 rpm for 20 minutes using a compufuge (Remi, India) and the supernatant was filtered through Whatman no. 4 filter paper. The filtrate was taken as crude enzyme extract and it was stored at-20°C.

Assay of PPO

PPO activity was assayed using the procedure of Oktay *et al*. (1995) with some modification. Briefly, 0.1 ml en-

Tab. 1. Independent variables and their coded levels used in RSM studies

Factors	Units	Level	
		Low (-1)	High (1)
A: Buffer Concentration of Extraction Buffer (BC)	mM	100	500
B: Buffer Concentration of Extraction Buffer (BC)		4.5	8.5
C: Extraction Time (ET)	Hours	1	12
D: Extraction Temperature (ETM)	°C	4	40
E: PMSF Concentration (PC)	mM	1	5
F: Volume of Extraction Buffer (VB)	ml	200	1000

100

Tab. 2. CCD design for six variables showing observed values of SA of PPO

Run	A: BC (mM)	B: PB	C: ET (Hour)	D: ETM (°C)	E: PC (mM)	F: VB (ml)	Response: SA Unit/mg protein
1	300	6.5	6.5	22	3	600	3235
2	300	6.5	6.5	22	3	600	3235
3	500	4.5	12	4	5	200	3105
4	500	4.5	12	40	1	1000	3112
5	100	4.5	1	4	5	200	3383
6	100	4.5	12	4	1	200	3309
7	500	8.5	12	4	5	1000	3007
8	300	6.5	6.5	22	3	600	3235
9	500	4.5	1	40	5	200	3107
10	100	8.5	12	40	1	1000	3381
11	500	8.5	1	40	1	1000	3017
12	100	8.5	12	40	5	200	3511
13	500	8.5	1	40	1	200	3047
14	500	4.5	12	40	5	200	3171
15	500	8.5	12	4	5	200	3103
16	500	4.5	1	4	1	1000	3043
17	500	4.5	12	4	1	200	3103
18	500	8.5	1	4	5	200	3077
19	300	6.5	6.5	22	3	600	3235
20	100	8.5	1	4	5	200	3305
21	500	4.5	12	40	5	1000	3157
22	500	4.5	12	40	1	200	3142
23	500	8.5	12	4	1	200	3077
24	100	8.5	12	4	1	200	3304
25	500	8.5	12	40	1	200	3103
26	100	8.5	1	40	5	1000	3357
27	500	4.5	12	4	1	1000	3077
28	100	8.5	1	40	1	200	3307
29	100	8.5	1	40	5	200	3441
30	100	4.5	12	4	5	200	3422
31	300	6.5	6.5	22	3	600	3235
32	500	8.5	1	4	5	1000	3043
33	100	8.5	12	4	5	200	3357
34	100	4.5	1	40	5	200	3493
35	100	4.5	1	40	5	1000	3422
36	500	4.5	1	4	1	200	3077
37	100	8.5	1	4	1	200	3269
38	500	4.5	1	4	5	200	3103
39	500	8.5	1	4	1	1000	3000
40	300	6.5	6.5	22	3	600	3235
41	100	4.5	12	40	1	1000	3441
42	100	8.5	1	4	1	1000	3263
43	100	4.5	1	40	1	1000	3333
44	300	6.5	6.5	22	3	600	3235
45	100	4.5	1	4	5	1000	3306
46	500	8.5	12	4	1	1000	3043
47	500	4.5	1	40	1	200	3103
48	100	4.5	12	40	1	200	3478
49	500	8.5	12	40	5	1000	3142
50	500	8.5	12	40	1	1000	3103
51	500	4.5	12	4	5	1000	3103

Tab. 2. CCD design for six variables showing observed values of SA of PPO (cont.)

Run	A: BC (mM)	B: PB	C: ET (Hour)	D: ETM (°C)	E: PC (mM)	F: VB (ml)	Response: SA Unit/mg protein
52	100	8.5	12	4	5	1000	3307
53	100	8.5	1	40	1	1000	3305
54	500	4.5	1	4	5	1000	3077
55	300	6.5	6.5	22	3	600	3235
56	500	4.5	1	40	5	1000	3125
57	500	8.5	1	40	5	200	3142
58	100	8.5	12	40	5	1000	3461
59	500	4.5	1	40	1	1000	3103
60	500	8.5	1	40	5	1000	3103
61	500	8.5	12	40	5	200	3157
62	100	4.5	12	40	5	1000	3550
63	100	4.5	12	40	5	200	3616
64	300	6.5	6.5	22	3	600	3235
65	300	6.5	6.5	22	3	600	3235
66	500	8.5	1	4	1	200	3043
67	100	4.5	12	4	1	1000	3305
68	100	4.5	1	4	1	1000	3271
69	100	4.5	1	40	1	200	3381
70	100	4.5	12	4	5	1000	3381
71	100	8.5	12	4	1	1000	3273
72	100	8.5	1	4	5	1000	3273
73	100	4.5	1	4	1	200	3304
74	100	8.5	12	40	1	200	3401

zyme extract was added to 2.9 ml of Catechol (100 mM) in 0.1 M phosphate buffer (pH-6.5) solution and change in absorbance at 420 nm was measured using a dual beam UV-Visible spectrophotometer (UV 3600, Shimadzu, Japan) against reference (3.0 ml catechol). Change in absorbance was recorded every 1 second for 3 minutes. One unit of PPO activity was defined as the change in absorbance of 0.001 per minute per milliliter of enzyme. Finally, enzyme activity was expressed in units/ml. Activity measurements were carried out in triplicate.

Measurement of specific activity of PPO

Protein quantity was estimated from the supernatant of each extract by the method of Lowry *et al*. (1951) and protein quantity was expressed in mg/ml using bovine serum albumin as standard. Specific activity (SA) of PPO was expressed in Units/mg of protein.

Mathematical modeling

Analysis of variance (ANOVA) was performed for the independent and dependent values to obtain regression equations that could predict the responses within a given range. The generalized second order regression equation used in the response surface study was as follows:

$$Y = \beta_0 + \sum_{i=1}^{6} \beta_i X_i + \sum_{i,j=1}^{6} \beta_{ij} X_i X_j + \sum_{i=1}^{6} \beta_{ii} X_i^2 \quad (1)$$

Where Y is the predicted response, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i, and X_j are the independent variables. For coded independent variables (A, B, C, D, E and F), the selected polynomial equation could be expressed as:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_5 E + \beta_6 F + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{15} AE + \beta_{16} AF + \beta_{23} BC + \beta_{34} CD + \beta_{45} DE + \beta_{56} EF + \beta_{11} A^2 \quad (2)$$

The design expert software was used to generate response surfaces and three dimensional (3D) plots. The adequacy and significance of the regression model was tested using ANOVA method. Test for significance on individual model coefficients and test for lack-of-fit was also estimated.

Determination of optimum extraction and validation of the final model

Optimum condition for the possible maximum extraction of PPO from potato peel depends on all the six parameters were obtained using the predictive equation of RSM. The software design expert was applied to search the optimum desirability of the response which is maximum SA of PPO. The verification of the validity and adequacy of the predictive extraction model with respect to all the six variables within the design space was done by performing a random set of 6 experimental combinations to study specific activity. Three verification run experiments were previously and remaining three experiments were those which have not been used but are within the range of the

levels defined previously. The experimental and predictive values of SA were compared to validate the model.

Results and discussion

ANOVA analysis

As summarized in Tab. 3, the ANOVA analysis of response 1: SA, the model F-value of 328.96 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" could be large which may occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, D, E, F, AB, AC, AD, AE, CD, DE are significant model terms. The insignificant model terms can be eliminated to improve the model. In this study, backward elimination procedure was used to reduced the insignificant terms . The predicted R^2 of 0.9775 is in reasonable agreement with the adjusted R^2 of 0.9832. The adjusted R^2 value corrects the R^2 value for the sample size and for number of terms used in the model. The high adjusted R^2 value (0.9832) obtained from ANOVA analysis indicating that the developed model is highly significant (Akhnazarova and Kafarov, 1982; Box *et al*., 1978). "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. From this study, the obtained ratio of 65.547 indicates an adequate signal. This model can be used to navigate the design space.

The model shows standard deviation (SD), mean, and predicted residual sum of squares (PRESS) value of 19.07, 3232.09 and 35513.75. Here, the calculated value of coefficient of variation (C.V %) is 0.59 and this lower value of C.V % designates a better reliability of the model (Khuri and Cornell, 1987). A corelation coefficient (R^2) of 0.9861 was obtained indicating high degree of corelation between the experimental parameters and response (SA of PPO in Unit/mg of protein).

The experimental results of the CCD design were fitted with a second order polynomial equation. The Eq. 3 depicts the empirical relationship between specific activity of extracted PPO (SA) and the six independent variables in coded units obtained by applying RSM.

$$SA = 3232.09 - 140.55 \times A - 21.58 \times B + 24.67 \times C + 40.61 \times D + 27.95 \times E - 16.52 \times F + 5.92 \times A \times B - 9.20 \times A \times C - 17.08 \times A \times D - 11.42 \times A \times E + 10.95 \times C \times D + 9.48 \times D \times E - 4.70 \times E \times F \quad (3)$$

While, the final empirical relationship between response:SA and the six independent process variables in actual units obtained by the applyication of RSM is given by Eq.4:

$$SA\ (U\ mg\ of\ protein^{-1}) = 3395.26878 - 0.55455 \times BC - 15.23047 \times PB + 4.56171 \times ET + 2.16974 \times ETM + 20.2743 \times PC - 0.023652 \times VB + 0.014805 \times BC \times PB - 0.00836648 \times BC \times ET - 0.00474392 \times BC \times ETM - 0.028555 \times BC \times PC + 0.11064 \times ET \times ETM + 0.26345 \times ETM \times PC - 0.00587891 \times PC \times VB \quad (4)$$

The normal probability described in Fig. 1. which shows some scatters along the line which indicates that the residuals follow a normal distribution. This designates that the model satisfies the assumptions of the ANOVA

Tab. 3. ANOVA table for response surface quadratic model (Response: SA of PPO)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1555515.953	13	119655.0733	328.9579291	< 0.0001	Significant
A-BC	1264219.141	1	1264219.141	3475.614522	< 0.0001	
B-PB	29799.39063	1	29799.39063	81.92503299	< 0.0001	
C-ET	38956.89063	1	38956.89063	107.1010005	< 0.0001	
D-ETM	105543.7656	1	105543.7656	290.1628624	< 0.0001	
E-PC	50008.14062	1	50008.14062	137.4833003	< 0.0001	
F-VB	17457.01563	1	17457.01563	47.99314855	< 0.0001	
AB	2244.390625	1	2244.390625	6.170320001	0.0158	
AC	5420.640625	1	5420.640625	14.90252494	0.0003	
AD	18666.39063	1	18666.39063	51.31798455	< 0.0001	
AE	8349.390625	1	8349.390625	22.95429833	< 0.0001	
CD	7678.140625	1	7678.140625	21.10888548	< 0.0001	
DE	5757.015625	1	5757.015625	15.82729328	0.0002	
EF	1415.640625	1	1415.640625	3.891905253	0.0531	
Residual	21824.38471	60	363.7397452			
Lack of Fit	21824.38471	51	427.929112			
Pure Error	0	9	0			
Cor Total	1577340.338	73				
Std. Dev.	19.07196228		R-Squared	0.986163807		
Mean	3232.094595		Adj R-Squared	0.983165966		
C.V. %	0.590080572		Pred R-Squared	0.97748504		
PRESS	35513.75454		Adeq Precision	65.54743972		

which depicting the accuracy and applicability of RSM in optimizing all six parameters to maximize the extraction of PPO.

Effect of experimental parameters on PPO extraction

The perturbation plot describes the comparative effect of all the parameters at the midpoint (coded 0.00) in the design space shown in Fig. 2. The most influential factor is characterized by a steep slope or curvature in a perturbation plot which shows that the response is very sensitive to that factor. In this study, perturbation plot suggests that all variables exerted different degree of quadratic effects. But the curve with the most significant change was the perturbation curve of variable A i.e. BC compared to those of the other factors fixed at their maximum levels. Thus, it is obvious that BC i.e. concentration of phosphate buffer was the most significant factor that contributed to the extraction of PPO and had the most pronounced quadratic effect.

The 3D response surface plots obtained from RSM study shows the interaction or combined effect of the variables on SA. As presented in Fig. 3(A), sharp increase in SA was observed with the decrease of both BC and PB when other parameters were kept as constant. Similarly, the value of SA gradually increases with the increase of PC and decrease of VB (Fig. 3B). Therefore lowering of volume and concentration of extraction buffer (Phosphate buffer) facilitates the extraction of PPO as value of SA increases. The reason may be that lowering of buffer concentration may facilitate the interaction between potato peel and extraction media whereas lowering volume of extraction buffer concentrates the enzyme. PMSF is serine protease

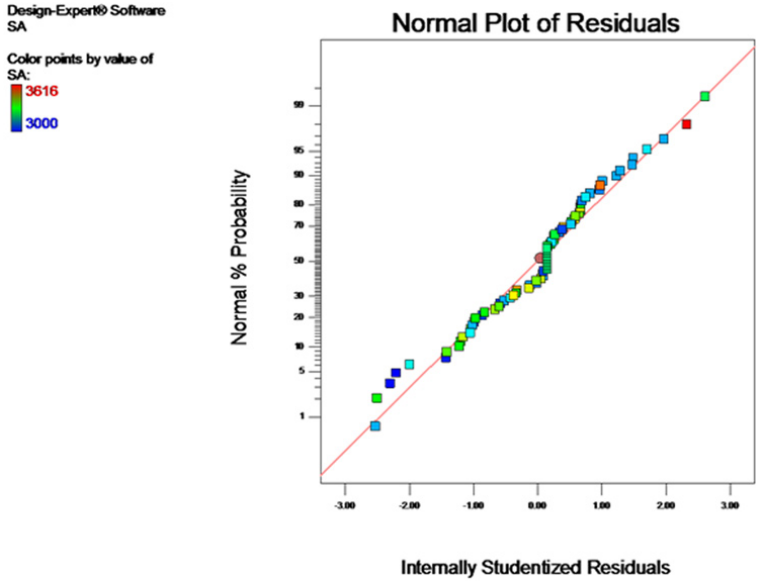

Fig.1. Normal plot of residuals for SA (Unit/ mg of protein)

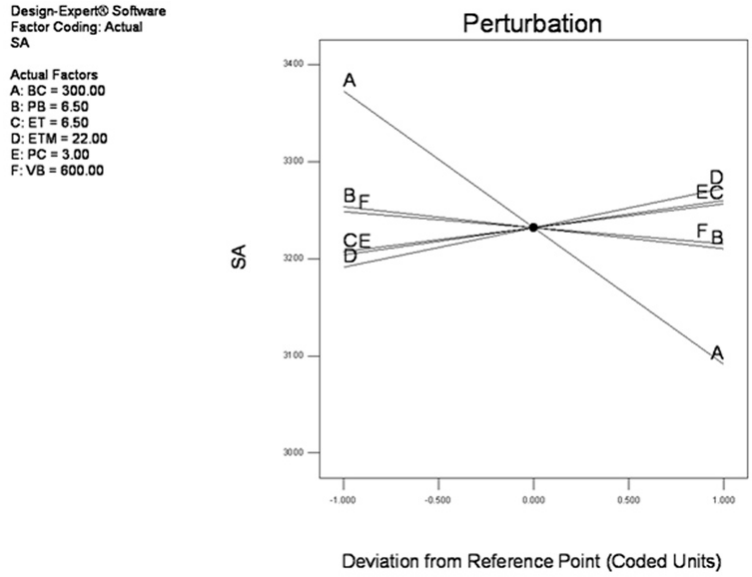

Fig. 2. Perturbation plot of independent process variables

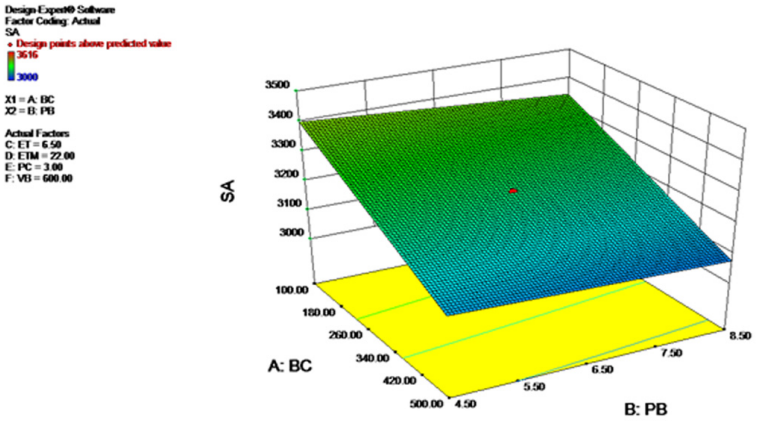

Fig. 3A. Three dimensional plots for the interaction effect of volume of extraction buffer (VB) and PMSF concentration (PC) on SA

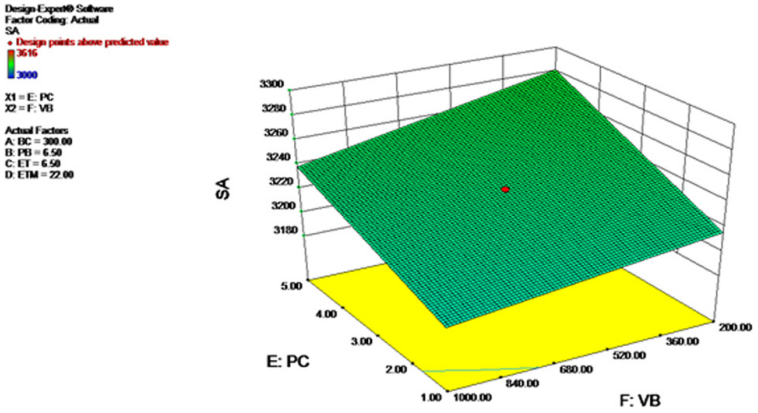

Fig. 3B. Three dimensional plots for the interaction effect of extraction time (ET) and temperature (ETM) on SA

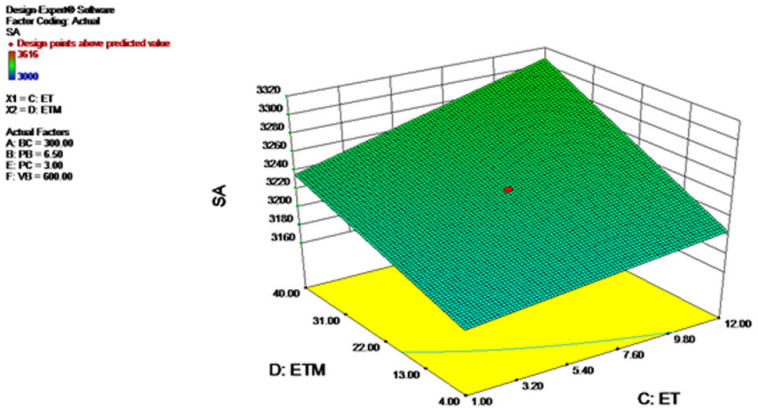

Fig. 3C. Three dimensional plots for the interaction effect of concentration of extraction buffer (BC) and p^H of extraction buffer (PB) on SA

inhibitor and increase concentration of this compound in the extraction buffer positively influence SA as it prevents the proteolysis of PPO by irreversibly blocking the serine residue of protease present in its active site (Gauillard and Richard-Forguet, 1997; Staszczak *et al*., 2000).

Significant increase in SA is observed with the increase of both ET and ETM (Fig. 3(C)). From Fig. 3(A), it is evident that extraction of PPO is positively influenced by acidic pH as SA increases with the decrease with pH. It may be due to the fact that acidic p^H may induce conformational change in the 3D structure of PPO which may results to expose the active site of PPO. Though this fact needs experimental proof, researchers have reported the allosteric behavior of PPO (Ricquebourg *et al*., 1996) and it has also been reported that PPO activity increases with increase in acidity (Valero *et al*., 1992).

Extraction of PPO is greatly influenced by time and temperature (Fig. 3(C)) because increase of extraction time prolongs the interaction between potato peels homogenate and extraction media. But, longer extraction

Tab. 4. Validation of the final reduced quadratic model

Parameters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Buffer Concentration (BC)	50	200	400	450	550	600
pH of Buffer (PB)	4	5	5.5	7	9	9.5
Extraction time (ET)	0.5	2	4	6	8	13
Extraction temperature (ETM)	2	6	12	24	36	42
PMSF Concentration (PC)	0.5	1.5	2	4	5.5	6
Volume of Buffer (VB)	50	100	250	500	700	1100
Predicted error (%)[a)]	0.07	0.09	0.146	0.304	0.90	0.058
Specific activity (SA) in Units/mg of protein						
Predicted	3323.5	3254.09	3139.19	3090.4	2978.26	2919
Actual	3320	3260	3130	3080	2970	2940
Predicted error (%)[a)]	0.10	0.18	0.29	0.33	0.26	0.72

Predicted error = (actual value-predicted value)× 100/predicted value

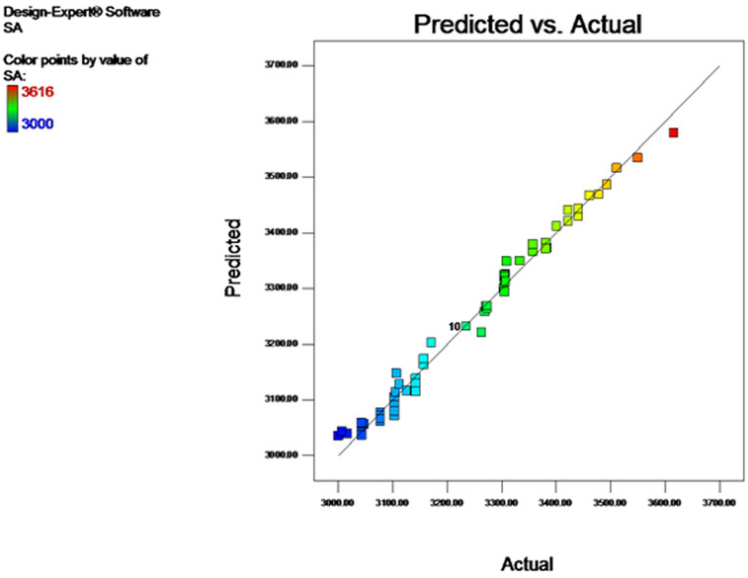

Fig. 4. Plot of predicted versus actual values of response (SA in Units/mg of protein)

period increases process economics and also chances of proteolysis. Rise in temperature increases SA of PPO by increasing the activity of PPO. So, the magnitude of all these parameters should be optimized to maximize the extraction of PPO.

Validation of developed model

Plot of experimental or actual value *vs*. predicted values of SA (given in Fig. 4) represents a high degree of similarity which designates the accuracy of the developed method. It also describes that the developed model satisfy the variance requirement and these also reflect applicability and accuracy of RSM for improved extraction of PPO.

The developed model was further validated by performing six additional experiments which constitutes three experimental combinations from the design and remaining three experiments were those which have not been used previously. As summarized in Tab. 4, experimental values were reasonably close to the predicted values confirming the validity and adequacy of the proposed model. Moreover, the validation experiments also proved that the predicted values of SA could be satisfactorily achieved within 0.80% of predicted error of experimental values.

Tab.5. Constraints for optimization of extraction conditions

Constraints			
Name	Goal	Lower Limit	UpperLimit
A:BC	Minimize	100	500
B:PB	Is in range	4.5	8.5
C:ET	Minimize	1	12
D:ETM	Is in range	4	40
E:PC	Is in range	1	5
F:VB	Minimize	200	1000
SA	Maximize	3000	3616

Optimization of extraction condition

In the present study, desirability function optimization of the RSM has been employed for the optimization of the extraction of PPO by means of the response; SA. The optimization module searches a combination of factor levels that simultaneously satisfies the requirements placed on

Tab. 6. Optimization result

Solutions No.	BC	PB	ET	ETM	PC	VB	SA	Desirability
1	100.00	4.50	1.00	40.00	5.00	200.00	3483.08	0.94104-Selected
2	100.00	4.57	1.01	40.00	5.00	200.57	3482.12	0.94017
3	100.03	4.50	1.15	40.00	4.98	211.58	3483.16	0.93443
4	100.02	4.50	1.00	40.00	4.71	211.60	3474.64	0.93348
5	101.55	4.50	1.00	37.48	5.00	209.51	3473.45	0.93262
6	100.11	4.50	1.09	39.71	4.27	200.11	3463.41	0.92928
7	100.01	6.13	1.00	40.00	4.66	200.12	3451.55	0.92525
8	100.06	6.86	1.03	39.70	5.00	200.03	3449.89	0.92372
9	101.87	4.50	1.13	30.00	4.99	200.43	3451.04	0.92115
10	101.02	4.50	1.02	32.66	3.77	200.11	3428.98	0.91238
11	100.00	4.96	2.41	40.00	5.00	200.00	3488.23	0.9118
12	100.00	7.98	1.00	39.18	4.73	201.25	3425.48	0.91129
13	100.00	7.86	1.00	38.82	4.67	200.00	3424.55	0.91114
14	100.00	4.67	1.00	32.53	3.49	200.00	3419.91	0.90865
15	100.03	4.50	1.00	28.83	3.75	200.02	3418.35	0.90775

each of the responses and factors in an attempt to establish the appropriate model. The aim of this optimization process was to find the optimum values of extraction parameters in order to maximize the value of SA in the extract. The constraints used during optimization process are summarized in Tab. 5. As one of the major aims of this study was to reduce process economics, the minimum level of two parameters viz. concentration and volume of extraction media (phosphate buffer) were used. Minimum value of extraction time was also taken to make the method less time consuming. The optimum experimental conditions required for maximum extraction of PPO from of potato peels are phosphate buffer concentration of 100 mm, buffer pH of 4.5, extraction time of 1 hour, 40°C temperature, PMSF concentration of 5 mM and buffer volume of 200 ml. The desirability of this optimization model is 94.10% (Tab. 6.) which is very much acceptable.

Conclusions

The CCD employed in this study proved to be an effective tool for the optimization of six influential process parameters to maximize the extraction of PPO. The best models were achieved by modified response surface model using backward elimination and these models provide good quality predictions for the six independent variables in terms of the extraction of PPO. The results of the present studies revealed that several factors influence the extraction of PPO and its activity. From the response surface analysis, BC had the most significant effect on SA among the six parameters studied. The results of ANOVA analysis which demonstrated optimal experimental conditions for extraction of PPO with maximum SA (3572.74 Units/mg of protein) were BC of 100 mm, PB of 4.5, ET of 1 hour, ETM of 40°C, PC of 5 mM and VB of 200 ml. This extraction model is cost effective and time saving as it requires low instrumental support. Further experiments including purification, characterization and immobilization of PPO from potato peel are currently under way.

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