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Mathematical Modeling and Simulation of Enzyme Assisted Bioactive Compound Extraction from Allium Cepa Using Response Surface Methodology

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Abstract **- In this present study, an attempt was made to investigate the enzyme assisted extraction process to extract the phenolic compounds from onion *(Allium cepa)* under various operating conditions such as temperature, extraction time and enzyme concentration. Three factors three level Box-Behnken response surface design (BBD) coupled with desired function methodlogy was used to optimize and model the extraction process. Optimum extracting conditions for the maximum phenloic compounds were determined and it was found to be: temperature of 40 ^{0}C, extraction time of 16 hour and enzyme concentration of 2 %. Under these conditions, 9.25 % of phenolic compound was extracted. Extracted phenolic compounds can be regarded as the promising antioxidants to scavenge the DPPH free radicals.**

Keywords: **Phenolic compounds, Onion, Enzyme assisted extraction, Modelling, Optimzation**

I. INTRODUCTION

Onions (Allium cepa) have been used for centuries in several societies against parasitic,fungal, bacterial and viral infections (Zou *et al.*, 2008). Chemical characterisation of sulphur compounds in onions has allowed stating that they are the main active antimicrobial agents.There are many techniques to recover antioxidants from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, microwave assisted extraction , ultrasound assisted extraction and enzyme assisted extraction. Among, various kind of extraction techniques, enzyme assisted extraction shows the advantages of non-toxic, maximum yield and reasanoble oprating cost over conventional extraction technique (Moure *et al.*, 2000). However, extraction yield and antioxidant activity of extract not only depend on the extraction process variables such as temperature, extraction time and enzyme concentration. Optimization of these variables will pay the way to attain the higher yield and antioxidant activity of extract. Even though the dynamic characteristics of the enzyme assisted etraction process is very complicated, a number of attempts in developing an experimental-based optimization methodology may help to provide a better understanding of the process in terms of the effects of independent variables and their interactions on the dependent variable (Missang *et al.*, 2005). Nowadays, optimization of extraction variables using stastical tool is emerging in excellent manner. One of the stastical tool used for optimization is response surface methodology (RSM) coupled with derringers desirability function method. The RSM is essentially a particular set of mathematical and statistical methods for designing experiments, building models, evaluating the effects of variables, and searching optimum conditions of variables to predict targeted responses (Ma *et al.*, 2009). The RSM does not require a large number of runs and also does not require too many levels of the independent variables. An extensive literature survey shows that, extraction of polyphenolic compounds from onion using enzyme assisted extraction via RSM in not yet described. Therefore, finding new and safe antioxidants from onion is of great interest for applications in natural antioxidants, functional foods, and neutraceuticals. The basic aim of the research was to determine the total phenolic content and its antioxidant activity in onion using enzyme assisted extraction. Moreover, Response surface methodology (RSM) coupled with three factor three level Box-Behnken response surface design (BBD) was used to optimize and investigate the extraction process variables such as temperature, extraction time and enzyme concentration (Liu *et al.*, 2010).

II. MATERIALS AND METHODS

A. Materials and Chemicals

The onion (*Allium cepa L.*) used in this study was purchased from Pungamuthur, Tamil, India. Cellulose enzyme was purchased from the local suppliers, Erode, India. Folin-Ciocalteu (FC) reagent, sodium carbonate anhydrous, gallic acid, sodium nitrite, sodium hydroxide, aluminum chloride anhydrous, 2,2- diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, were purchased from Sigma-Aldrich GmbH (Chennai, India). All other chemicals and reagents used were of analytical grade.

B. Extraction of Phenol Compounds

The fresh raw onions were cut into smaller pieces (1 cm x 1 cm x 1 cm), weighed and added to distilled water with the solid:water ratio of 2:1. Then, the mixture of sample was

stirred on the hot plate for 10 minutes, and allowed to cool down to room temperature. The pH was adjusted to pH 7 by using 0.1N NaOH and 0.1N HCl. The cellulose enzyme with different concentrations were added and the samples were extracted at different periods of time and temperature with constant shaking (200 rpm). After that, the samples were centrifuged (10,000 rpm, 30°C) for 10 minutes and the supernatants were collected, filtered into a beaker. Finally, the extracts were dried in a drying oven at 60°C and it is used for determination of phenolic content.

C. Determination of Total Phenolic Contents in the Onion Extracts

The concentration of phenolics in extracts was determined using spectrophotometric method. Based on the measured absorbance, the concentration of phenolics was read the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of onion).

D. Evaluation of Antioxidant Activity

The ability of the onion extract to scavenge DPPH free radicals was assessed using antioxidant activity. The percentage inhibition of radicals was calculated using the following formula (Li et al., 2010)

$$Y = \frac{A_{Control} - A_{Sample}}{A_{Sample}} * 100 \quad (1)$$

where Y is inhibition (%). $A_{control}$ is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. The data were presented as mean values ± standard deviation (n = 3).

E. RSM Design

In this present study, response surface methodology (RSM) coupled with Box-Behnken response surface experimental design (BBD) was employed to investigate the individual and interactive effects of process variables on the extraction of phenolic compounds from onion. Temperature (A), extraction time (B) and enzyme concentration (C) are selected as independent variables, whereas extraction of phenolic content (Y) is selected as response. Twenty nine experiments were designed with five replications. An empirical second-order polynomial model (Y) (response function) for predicting the optimal point was in the following form:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} X_i X_j + e_i \quad (2)$$

Adequcy of developed mathematical models were investigated by the pareto analysis of variance (ANOVA) and developed models was used to plot the response surface contour graphes in order to study the interactive effect of independent variables on the responses All the statistical analysis was done using Design- Expert 8.0.7.1 (State-Ease Inc., Minneapolis, MN, USA) package. The detailed methology used in this study was reported elsewhere (Kumaran et al., 2006).

III. RESULTS AND DISCUSSION

A. Mathematical Modelling

Process variables and their ranges are shown in Table I. For the investigation of the effect of extraction conditions on the phenolic content, batch experiments were performed according to BBD design and the results are tabulated in Table II. Different response functions such as linear, interactive, quadratic and cubic models were used to correlate the experimental data and to obtain the regression equation. To decide about the adequacy of the model three different tests namely sequential model sum of squares and model summary statistics were carried out in the present study and the results are presented in Table III. Experimental data were used for determining of the response function coefficients for each independent variable (Ismail *et al.,* 2010). The response function with the determined coefficients for the extraction yield of phenolic content as follows

$$Y = 8.96 - 0.36A + 0.21B + 0.73C + 1.79AB - 0.92AC - 1.09BC - 2.26A^2 - 0.96B^2 - 1.21C^2 \quad (3)$$

Where, Y is extraction yield of phenolic content (%); A, B and C are temperature, extraction time and enzyme concentration respectively.

The statistical significance of the response function was checked by F-test, and the ANOVA results for response surface quadratic model and model terms are summarized in Table 4. The model F value and very low probability value (0.0001) indicated that the model was statistically significant and model equation can adequately be used to describe the response. Adequcy of the developed mathematical model examined as follows, a normal distribution function was then fit to the studentized residuals. Then, the studentized residual predicted by the best-fit normal distribution was plotted against the experimentally obtained studentized residual in Fig.1a. Fig. 1b plots was the studentized residuals versus predicted. This plot is expected to be a random scatter, indicating that the variation in the original observations is not related to the value of the response and it further indicates that the suggested model is an appropriate description of the process. Further, diagnostic plots such as predicted versus actual (Fig. 1c) help us to evaluate the model suitability and find out the relationship between predicted and experimental values. The data points on this plot lie reasonably close to the straight line and indicate that an adequate agreement between real data and the data obtained from the model (Gutteridge *et al.,* 2007).

B. Effect of Process Variables on the Extraction of Phenolic Compounds

The regression model (Eq. (3)) can be used to predict the enzyme assisted extraction of phenolic compounds (response) for the different values of the tested variables and to identify the major interactive effects between the test variables from the circular or elliptic nature of the contours. The 3-dimensional response surfaces were plotted based on Eq. (5) and these response surfaces by computer modeling show that the center point derived from one-by-one variations (Fig.2).

1. Effect of Temperature

Temperature is one of the crucial extraction parameter in the extraction of phenolic compounds from onion using enzyme assisted extraction. In order to investigate the effect of temperature on extraction yield of phenolic compounds, experiments were carried out in various temperature (30-50 ^{o}C) and the results are shown in Fig.2a-b. From the results, it is observed that, the extraction yield of phenolic compound is increased linearly with increasing temperature upto 45 ^{o}C. However, temperature beyond 45 ^{o}C shows negative impact on extraction yield of phenolic compounds (Guo *et al.,* 2011).

2. Effect of Extraction Time

Extraction time is one of the key parameter which influences the enzyme assisted extraction significantly. To exmine its effect on the extraction yield of phenolic compound, experiments were carried out in various extraction time (10-24 hours) and the results are shown in Fig. 2. From the Fig.2b-c, it is found that, the extraction yield of phenolic compound is increased rapidly with increasing the extraction time upto 18 hour. Beyond 18 hour of extraction time shows the negligible effect on the extraction yield of phenolic compound from onion (Gong *et al.,* 2012).

3. Effect of Enzyme Concentration

Enzyme concentration is a important parameter influences the extraction yield of polyphenloic compound from onion using enzyme assisted extraction. So that, experiments were carried out to study the effect of enzyme concentration (0.8-2.8 %) on the extraction yield of phenolic compound. From the results (Fig. 2a-c), it is observed that, the extraction yield of phenolic compound is increased linearly with increasing enzyme concentration from 0.8 to 2 %. However, enzyme concentration beyond 2 % resulted in negligible effect on the extraction yield of phenolic compound (Falleh *et al.,* 2008).

C. Multi Response Optimization and Validation

After analyzing the polynomial equation depicting the effect of independent variables on the response, optimization process was carried out. From the results, it is found that optimum condition for the maximum yield of phenolic compound found to be as follows: temperature of 40 ^{0}C, extraction time of 16 hour and enzyme concentration of 2 %. Under these conditions, 9.25 % of phenolic compounds was extracted and it is validated (9.18 %) used by conducting the additional experiment in the above mentioned opimum conditions in triplicates.

D. Antioxidant activity of extracted phenolic compound

Fig.3 shows the DPPH scavenging activities of the extracts in a concentration-dependent manner. The extract obtained by 100% ethanol yielded the highest DPPH radical scavenging activity at concentration range of 100 µg/mL. However, at concentrations ranging from 175 µg/mL to 200 µg/ mL, its DPPH radical scavenging activity is not significantly different from those of the other extracts (Chen et al., 2011). The IC_{50} of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%, which is obtained by interpolation from a linear regression analysis. A lower IC_{50} value indicates a higher antioxidant activity of a the extracted compound (Chen *et al.,* 2008).

TABLE I PROCESS VARIABLES AND THEIR RANGES

Level	**-1**	**0**	**1**
Temperature (^{o}C)	30	40	50
Extraction time (hour)	10	17	24
Enzyme concentration (%)	0.8	1.6	2.4

TABLE II BBD DESIGN OF RESULTS

S.No.	**A**	**B**	**C**	**Y**
1	40	17	1.6	8.96
2	40	17	1.6	8.96
3	40	17	1.6	8.96
4	50	17	0.8	5.28
5	50	10	1.6	3.62
6	30	17	2.4	7.54
7	40	24	2.4	6.84
8	50	17	2.4	4.58
9	40	10	0.8	4.55
10	40	17	1.6	8.96
11	30	17	0.8	4.58
12	40	17	1.6	8.96
13	40	10	2.4	8.54
14	30	10	1.6	7.52
15	30	24	1.6	4.28
16	50	24	1.6	7.54
17	40	24	0.8	7.22

TABLE III SEQUENTIAL MODEL SUM OF SQUARES FOR RESPONSES

Source	Sum of Squares	Df	Mean Square	F Value	Prob > F	Remarks
Sequential model sum of squares for total phenolic content						
Mean	803.72	1.00	803.72			
Linear	5.70	3.00	1.90	0.44	0.7278	
2FI	20.94	3.00	6.98	1.99	0.1797	
Quadratic	34.54	3.00	11.51	142.29	< 0.0001	Suggested
Cubic	0.57	3.00	0.19	63660000.00	< 0.0001	Alised
Residual	0.00	4.00	0.00			
Total	865.46	17.00	50.91			
Model summary statistics						
Model	Std.Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Total phenolic content						
Linear	2.0763	0.0923	-0.1172	-0.5608	96.4	
2FI	1.8736	0.4314	0.0903	-0.5110	93.3	
Quadratic	0.2844	0.9908	0.9790	0.8532	9.0620	Suggested
Cubic	0.0000	1.0000	1.0000		+	Aliased

TABLE IV ANOVA RESULTS FOR RESPONSE

Source	Sum of Squares	df	Mean square	F value	P-value
Model	61.18	9.0000	6.79727	84.0095	< 0.0001
A	1.05	1.0000	1.05125	12.9927	0.0087
B	0.34	1.0000	0.34031	4.20603	0.0794
C	4.31	1.0000	4.30711	53.2329	0.0002
AB	12.82	1.0000	12.8164	158.402	< 0.0001
AC	3.35	1.0000	3.3489	41.3901	0.0004
BC	4.77	1.0000	4.77423	59.0061	0.0001
A^2	21.43	1.0000	21.4344	264.914	< 0.0001
B^2	3.91	1.0000	3.9108	48.3347	0.0002
C^2	6.15	1.0000	6.1519	76.0332	< 0.0001
CV	4.14				
AP	20.56				
PRESS	12.02				
Mean	6.58				
R^2	0.9868				
Adj-R^2	0.9699				

IV. CONCLUSION

In this present study enzyme assisted extraction of phenolic compounds was carried out to optimize and determine the effect of various parameters such as temperature, extraction time and enzyme concentration. RSM coupled with BBD was employed to study and optimize the process variables on the extraction of phenolic compounds. From the results, it was observed that all the process variables have significant effects on the phenolic compound extraction and the quadratic model was developed for predicting the response. The highest phenolic compound yield (9.25%) was achieved at the optimum conditions as follows: temperature of 40 ^{0}C, extraction time of 16 hour and enzyme concentration of 2 %. The DPPH radical scavenging activity of extracted phenoilc compound is highly significant.

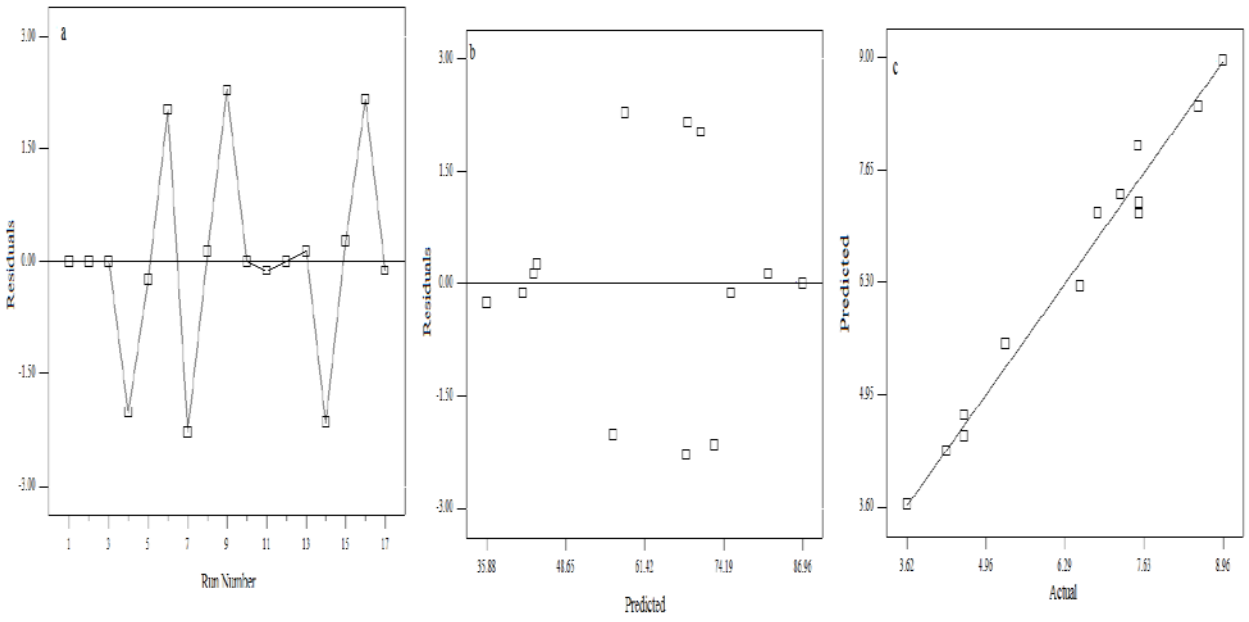

Fig.1 Model Adequacy Plots

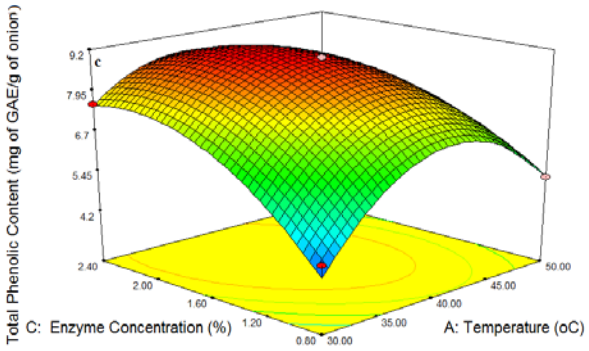

Fig. 2 Response Surface Plots representing the effect of Process Variables on total Phenolic content.

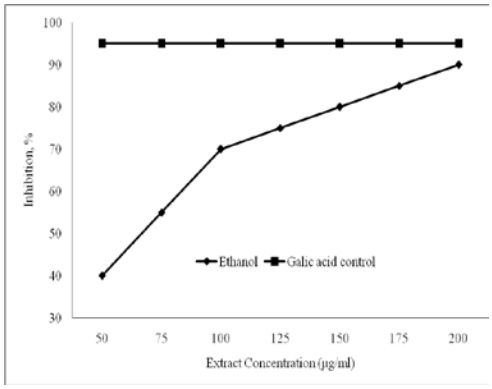

Fig.3 DPPH Scavenging Activity of the Extract.

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