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EFFECT OF CRUDE ENZYME, INCUBATION TIME AND TEMPERATURE ON THE JUICE RECOVERY AND QUALITY FROM ALU BUKHARA (*PRUNUS DOMESTICA L.*)

Robin*

Sanjay Kumar*

Dhir Singh**

H. K. Sharma*

Abstract: *The effect of incubation temperature (35-55°C), incubation time (210-540 min), and crude enzyme (0.05-0.15 ml/50g pulp) concentration on juice yield, viscosity and clarity of juice was determined. The establishment of the optimum conditions for enzymatic hydrolysis of alu Bukhara to obtain maximum juice yield, clarity and minimum viscosity were obtained using central composite rotatable design. The changes in juice yield, viscosity and clarity of juice with respect to hydrolysis parameters were established with the coefficient of determination, R^2=0.9824, 0.9786 and 0.9769 for juice yield, viscosity and clarity of juice respectively was described by the significant regression model. Incubation time was the most significant variable affecting the juice yield whereas viscosity and clarity of juice were most significantly affected by the concentration of crude enzyme. The study recommended enzymatic treatment conditions were: incubation time 463min, incubation temperature 45°C, and crude enzyme concentration 0.12 ml/50g alu bukhara pulp.*

Keywords: *Alu bukhara juice; Enzyme treatment; Optimization; Crude enzyme*

*Sant Longowal Institute of Engineering and Technology (Deemed to be University),

**Central Food Laboratory, Mumbai

INTRODUCTION

Alu bukhara (*Prunus domestica L.*) belongs to the *prunus* genus of plants and are relatives of the peach, nectarine, plum and almond. The alu bukhara fruit is a good source of vitamins, minerals, fiber and enzymes that are good for the digestive system and helps in maintaining balanced nutrition and can be eaten raw or used to make juice and other products.

Alu Bukhara fruit also has potential to contribute greatly to human nutrition because of their richness in fibre and antioxidants [1]. Neochlorogenic and chlorogenic acid, two dominant phenolic compounds in prunes, were antioxidants toward isolated human LDL [2]. Consuming peaches, plums and nectarines is positively associated with nutrient intake, improves anthropometric measurements and reduced risk of hypertension [3]. Despite reports of plum benefits to human health, consumption remains low, which has been attributed to a lack of fruit ripening before consumption [4]. Alu bukhara contains high amounts of secondary plant metabolites mainly polyphenols, featuring a high antioxidant capacity. They also contain considerable amounts of fruit acids, which normally prevent the marketing of 100% natural juices.

Generally, three methods of juice extraction are employed viz, cold, hot, and enzymatic methods . The use of fungal enzyme in fruit juice extraction had shown significant increase in juice recovery as compared to cold and hot extraction methods [5]. The enzymes, mainly pectinases, and cellulases assist in pectin and cellulolytic hydrolysis respectively, which cause a reduction in pulp viscosity and a significant increase in juice yield [6]. The extraction of plum juice on large scale bases includes pressing of juice from comminuted solids of plum. The residual pulp remaining after juice extraction still contains valuable extractable material such as particulate, flavor, soluble solids, etc., which would improve the final quality of the juice. By adding cell wall liquefying enzymes, it is possible to further extract valuable juice components from pulp.

The enzymatic hydrolysis of pectic substances depends on several processing variables such as type of enzyme, hydrolysis time, enzyme concentration, incubation temperature, and pH [7]. These parameters need to be optimized for maximal juice recovery but the cost of the processing becomes a limiting factor in the application of commercial enzymes therefore the present study was undertaken to use crude enzyme from *A. Niger* for the treatment of the alu bukhara pulp to improve the juice yield with optimum overall acceptability.

Therefore the objective of the present study was undertaken to optimize the hydrolysis pretreatment parameters (incubation temperature, time of treatment and concentration of crude enzyme) for the maximal juice yield from alu bukhara with optimum quality.

MATERIALS AND METHODS

2.1 Materials

Fully ripe fresh alu bukhara (*Prunus domestica L*) without any visual blemishes were purchased from local market of Sangrur, Punjab, India. The fruits were washed, cut with the help of knife and were ground (Sujata mixer grinder, New Delhi) to make pulp. The fruit pulp so prepared was used to extract juice.

2.2 Crude enzyme preparation

Aspergillus niger NCIM 548, obtained from the national chemical laboratory, Pune, was utilised for pectinase production, since this mould produced a good amount of cellulose and pectinase and hemicellulose too. The organism was maintained on potato dextrose agar slant and sub-cultured every 7-8 weeks. It was used for the production of crude enzyme under solid state fermentation (SSF) using wheat bran, corn bran and kinnow peel (in 2:1:2 ratio) medium [8]. The other ingredients were: $(NH_4)_2SO_4$– 1.0, $MgSO_4$– 5.0, $FeSO_4.7H_2O$ – 0.005 and KH_2PO_4 -5.0 [9] gl^{-1}. Medium so prepared was autoclaved for 15min at 120° C. These flasks were then incubated at 30°C for 11 days, the solid state fermentation (SSF) conditions optimized for the maximum production of both pectinase and cellulase using *Aspergillus Niger* NCIM 548. The medium obtained was first filtered by double folded cheese cloth to filter wheat bran than followed by a membrane having pore size of 40µm using vaccum filteration device. The filtrate was centrifuged at 10,000 rpm for 10 min in centrifuge (Model r8c, Remi Equipments Ltd, India) to remove all cell mass. The supernatant was lyophilized to concentrate the obtained crude enzyme. The lyophilized enzyme was than checked for cellulase and pectinase activity [10] and protein content [11] and was filtered through 0.2µm syringe filter to remove the spores of the fungus to avoid the microbial contamination of the juice. This filtered enzyme containing 21 U/ml of the pectinase and 8 U/ml of the cellulase was then used for the treatment of alu bukhara pulp to improve the juice yield and quality.

2.3 Experimental design and statistical analysis

Response surface methodology (RSM) was adopted for designing experiment as it

emphasizes the analysis and modeling of the problem in which response of interest is influenced by several variables and the objective is to optimize this response [12]. The biggest advantage of RSM is the reduced number of experimental runs needed to provide enough information for statistically acceptable results. A five-level three-factor central composite rotatable design was executed [13]. The independent variables were the temperature of enzyme treatment (X1), time of treatment (X2), and used enzyme concentration (X3). The variables and their levels were selected based on the literature available on enzymatic hydrolysis of guava [14]. These were the Incubation temperature (X_1; 35 – 55 °C), time (X_2; 210 – 540 min) of the enzymatic treatment, and concentration of crude enzyme used (X_3; 0.05 – 0.15 ml/50 g pulp). The pH of the pulp was kept at its natural value (4.0–5.2) and was not included in the RSM experimental design as the pH range is optimal for the exogenous pectinases [15]. The three independent variables were coded as −1.682 (lowest level) −1, 0, 1 and +1.682 (highestlevel). The experimental design matrix in coded (x) form and at the actual level (X) of variables is given in Table 1. The experimental design matrix in coded (x) form and at the actual level (X) of variables is given in Table 3. The response function (Y) was related to the coded variables by a second degree polynomial equatin (Eq. 1) as given below:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x^2_1 + b_{22} x^2_2 + b_{33} x^2_3 + \varepsilon \quad \text{.....(1)}$$

The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23}, (interaction effects); b_{11}, b_{22}, b_{33} (quadratic effects); and ε (random error).

2.4 Analysis of response variables:

2.4.1 Enzymatic treatment and juice yield

For each experiment, 50 g of pulp was used to different enzyme treatment conditions, as shown in Table 2. The incubation temperature was adjusted to the desired level (±0.5°C) by using a high precision water bath (Seco, Model 129, India). At the end of the enzyme treatment, the suspended pulp was filtered through 6 times folded cheese cloth and the extracted juice was heated at 90 °C for 5 min to inactivate the enzyme [16] using the same water bath. The juice thus collected was considered as clear juice. The juice yield was then calculated using the following expression:

Juice yield, % = $\frac{\text{Weight of clear juice}}{\text{Weight of sample}}$ *100

2.4.2 Clarity

Juice clarity was measured according to the **methods of crop [17] and ough [18]**. The juice was shaken and 10 ml of it was centrifuged at 3000 rpm for 10 min to remove pulp and cloud particles. The clarity of the juice obtained was measured by measuring the transmittance at a wavelength of **570 nm** using UV- VIS spectrophotometer (UV 5704SS, Electronics Corporation of India Ltd.). Distilled water was used as a reference. The percent transmittance was considered as a measure of juice clarity.

2.4.3 Viscosity

Clean and dried Ostwald capillary viscometer was used for determining the viscosity of juice. Double distilled water was used as a reference. The Time taken to flow through the capillary section of the Oswald viscometer was noted using a stopwatch for the reference and the sample at 20 $\pm$ 2^{o}C [19].

Apparent viscosity $\frac{\eta}{\eta_w} = \frac{D_S \text{ x } t_s}{D_W \text{ x } t_w}$

Where,

D = density

t = time of flow

s = sample

w = water.

2.5 Optimization and validation of the model

The obtained data was graphically analysed by using Design Expert version 6.0.10 (Trial version; STAT-EASE Inc., Minneapolis, MN, USA) software. The optimum values of the selected variables were analyzed by the response surface contour plots and also by solving the regression equation. Experimental analysis of suggested optimum conditions by the design expert was done to predict the validity and adequacy of the predictive models.

RESULTS AND DISCUSSION

The juice yield (%), viscosity and clarity of the extracted juice from enzyme treated and untreated (control) pulp was evaluated. The range of parameters (juice yield, apparent

viscosity and clarity) of enzyme treated and control samples are shown in Table 2. The data showed that the quality and quantity of extracted juice has been improved significantly by the enzymatic treatment. The experimental values for all the three responses (juice yield, apparent viscosity and clarity) under different combination of treatment conditions are given in Table 3.

3.1. Fitting the model

The coefficient of determination (R^2) provided the judgement for the adequacy and fitness of the model. The closer the value of R^2 value unity, the better is the empirical model fits the actual data. The coefficients of determination, R^2, defined by the model were 0.9824, 0.9786 and 0.9769 for the regressed models predicting the juice yield, viscosity and clarity respectively (Table 4), suggesting a good fit for the models. The defined models seemed to be reasonably represent the observed values. Thus, the responses were sufficiently explained by the model.

The adjusted R^2 was a corrected value for R^2 after elimination of the unnecessary model terms, which was very close to their corresponding R^2 values for all the responses. Higher values of adjusted R^2 also advocated significance of the models. The coefficient of variation (CV) explains the extent to which the data are dispersed and is a measure of residual variation of the data relative to the size of the mean; the small values of CV give better reproducibility. The small CV values 1.77, 3.15 and 5.56 of the responses juice yield, viscosity and clarity respectively (Table 4), explained that the experimental results were precise and reliable.

The F-value of 61.92, 50.88 and 47.04 for juice yield, viscosity and juice clarity, respectively (Table 4) concluded that the models were significant ($P< 0.001$). The model for the juice yield, viscosity of juice and clarity can be designed by the coefficients for the predictions of the results.

3.2. Response surface analysis

3.2.1 Juice yield

Table 3 shows the juice yield under different experimental conditions ranged 58.1 to 79.6%. The minimum juice yield was obtained under crude enzyme concentration; 0.10 ml/50g, incubation time; 375 min and temperature; 28.18°C whereas maximum juice yield was

observed at crude enzyme concentration, 0.10 ml/50g, incubation time, 375 min and temperature, 45°C.

The response surface graphs were plotted to determine the optimum level of each variable and explained the interaction of the variables. The response surface curves of juice yield are shown in Figures 1a-b. Each figure explains the effect of two factors while the third factor was fixed at middle level. Figure 1a is the response surface curve of variation in the juice yield as function of incubation temperature (X_1) and incubation time (X_2), fixing the concentration of crude enzyme(X_3) at middle level i.e. 0.10 ml/50g of plum respectively. The figure shows that the juice yield increased with the increase in both time and temperature. With further increase in temperature above 45.77°C and incubation time beyond 516.42 min, the juice yield decreased slowly. The decrease in juice yield with increasing temperature beyond 45.77°C may be due to denaturation of protein which leads to decrease in enzyme activity at higher temperature. The results are supported by the findings of [20], who reported that the maximum juice yield from guava is obtained by pectinolytic enzyme treatment of pulp at 43.3°C temperature for 447 min of time.

Fig. 1b depicts the interactive effect of concentration of crude enzyme (X_3) and incubation temperature (X_1) to juice yield. The data shows that the juice yield increased with increase in temperature and concentration of crude enzyme up to 45.77°C of temperature and 0.13 ml of crude enzyme concentration. The juice yield decreased slowly beyond 45.77°C temperature, it may be due to decrease in enzyme activity at higher temperature. The increase in juice yield with increasing pectinase enzyme concentration is also supported by [6] who reported that pectinases degrade pectic substances leading to increase in juice yield.

3.2.2 Viscosity

The results showed that the viscosity of juice ranged from 2.41 to 1.49 cps (Table 3). The viscosity of juice was minimum when the experimental condition, temperature, time and concentration of crude enzyme were 45°C, 375 min and 0.10 ml/50g of pulp respectively whereas it was observed maximum with 61.82°C temperature, 375min of time and 0.10 ml/50g of crude enzyme concentration (Table 3).

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable (Fig 2a-b). Figure 2a is the response surface

curve of incubation temperature (X_1) and incubation time (X_2) on viscosity of juice keeping the other factor at its middle level. It is clear from the figure that with increase in temperature and time the viscosity decreased up to 44.35°C and 400.00 min. With further increase in temperature beyond 44.35°C, the viscosity of juice increased. The increase in viscosity with increasing temperature may be due to inactivation of enzyme at higher temperature. The findings are in accordance with [21] who reported that the viscosity of the banana juice decreases with increase in temperature of the enzymatic treatment reaction up to 42°C. The temperature increased the rate of enzymatic reactions. Upon enzyme treatment, degradation of pectin leads to a reduction of water holding capacity and consequently free water was released to the system thus reducing the viscosity of the juice.

Figure 2b, depicts the interaction effect of incubation temperature (X_1) and crude enzyme concentration (X_3) to viscosity. The figure shows that the viscosity decreased with increase in concentration of crude enzyme and incubation time. The viscosity of juice decreased up to 0.15 ml/50g of pulp of crude enzyme concentration and 44.35°C temperature. The juice viscosity increased with further increase in temperature. [22] reported the increase in viscosity of the blended carrot-orange juice with increase in temperature beyond 50°C. [21] observed that the viscosity of the juice decreases with increase in enzyme concentration up to its maximum value (0.1%).

3.2.3 Juice clarity

Table 3 depicts that the clarity of the juice ranged from 6.3 to 15.05%T (Table 3). The minimum clarity was 6.3%, when the pulp was treated with 0.05ml/50g, crude enzyme concentration for 210min time at 35°C temperature whereas the maximum clarity was observed at crude enzyme concentration; 0.10 ml/50g, time; 375 min and temperature; 45°C.

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable (Fig 3a-b). Fig. 3a shows the effect of incubation temperature (X_1) and time (X_2) on juice clarity. It was evident from the figure that, clarity of juice increased with the increase in both time and temperature up to 540 min and 46°C respectively. With further increase in temperature, the clarity of juice decreased. [22] observed that the clarity of the blended carrot-orange juice decreased with increase in temperature beyond 50°C. The clarity of juice (Fig. 3b) increased with the increase in both

concentration of crude enzyme and incubation temperature up to 0.13 ml/50g pulp and 46.50^{o}C. Degradation of the polysaccharides like pectin leads to a reduction in water holding capacity and consequently, free water is released to the system which increases the yield and clarity of juice [23]. With further increase in the incubation temperature the clarity of juice decreased.

3.3. Optimization and verification of process variables

Maximum possible juice yield and clarity and minimum viscosity of juice were the main criterion for constraints. Under these constraints, the optimum treatment conditions were obtained were: incubation temperature, 45.24°C, incubation time, 462.69 min and concentration of crude enzyme, 0.12 ml/50g pulp (Table 5). But in actual practice, the recommended conditions were difficult to maintain during processing and some deviation is expected. That is why the optimum conditions were targeted as temperature, 45^{o}C, time, 463min and concentration of crude enzyme, 0.12 ml/50g pulp. Under the targeted conditions (constraints), experiments were conducted to find the variation in juice yield, viscosity and clarity of juice. The experimental values of conducted experiments were very close to the predicted values (Table 5) with a desirability of 0.988 and the deviation in maximum percentage was 3.09. It shows that there was a high fit degree between the observed and predicted values from the regression model.

CONCLUSIONS

The present study revealed that plum juice yield, viscosity and clarity are function of enzymatic hydrolysis conditions. Significant regression model describing the variation of juice yield, viscosity and clarity with respect to the independent variables, temperature, time and concentration of crude enzyme was established. Incubation time was the most significant variable affecting the juice yield whereas viscosity and clarity of juice were most significantly affected by the concentration of crude enzyme. The recommended enzymatic treatment conditions from the study were: incubation time 463 min, and incubation temperature 45°C, and crude enzyme concentration 0.12 ml/50g plum pulp.

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Table 1. Experimental range and levels of the independent variables

Variables	Range and levels				
	-1.682	-1	0	1	1.682
Temp. (X_1, ^oC)	28.18	35	45	55	61.82
Time (X_2,min)	97.50	210	375	540	652.50
Conc. of crude enzyme (X_3, ml)	0.02	0.05	0.10	0.15	0.18

Table 2: The range of different parameters (Juice Yield, Apparent viscosity and Clarity) of juice obtained from untreated and enzyme treated plum pulp

Parameter	Units	Untreated	Enzyme treated
Juice Yield	% w/v	56	58.1 - 79.6
Juice apparent Viscosity	Cps	4.23	2.41-1.49
Juice Clarity	% T	0.8	7.7 - 15.05

Table 3. The central composite rotatable experimental design employed for enzymatic hydrolysis pretreatment of plum

Exp. No.	Coded Variables			Independent Variables			Responces		
	X_1	X_2	X_3	Temp. (^oC)	Time (min.)	Conc. of Crude Enzyme(mg)	%age Yield	Viscosity (cps)	Clarity (%T)
1	0	0	0	35	210	0.05	62.1	2.07	7.7
2	+1	-1	-1	55	210	0.05	62.6	2.17	9.1
3	-1.682	0	0	35	540	0.05	71.2	1.99	8.4
4	0	0	0	55	540	0.05	71.8	2.11	8.61
5	+1	+1	-1	35	210	0.15	71	1.81	9.8
6	0	0	0	55	210	0.15	73.2	1.95	10.5
7	-1	-1	+1	35	540	0.15	72.4	1.86	11.9
8	0	0	0	55	540	0.15	74.3	1.91	13.3
9	-1	+1	+1	28.18	375	0.1	58.1	2.33	7.7
10	2	-1.682	0	61.82	375	0.1	61.2	2.41	9.8
11	0	0	+1.682	45	97.50	0.1	64.8	2.13	7
12	0	0	-1.682	45	652.50	0.1	76.8	2	11.2
13	-1	-1	-1	45	375	0.02	60.3	2.3	6.3
14	0	0	0	45	375	0.18	73.6	1.61	12.6
15	-1	+1	-1	45	375	0.1	77	1.58	13.79
16	0	+1.682	0	45	375	0.1	76.8	1.61	14.7
17	+1	-1	+1	45	375	0.1	77	1.53	14.7
18	+1	+1	+1	45	375	0.1	79.6	1.49	14.91
19	+1.682	0	0	45	375	0.1	76.2	1.53	14
20	0	0	0	45	375	0.1	79	1.55	15.05

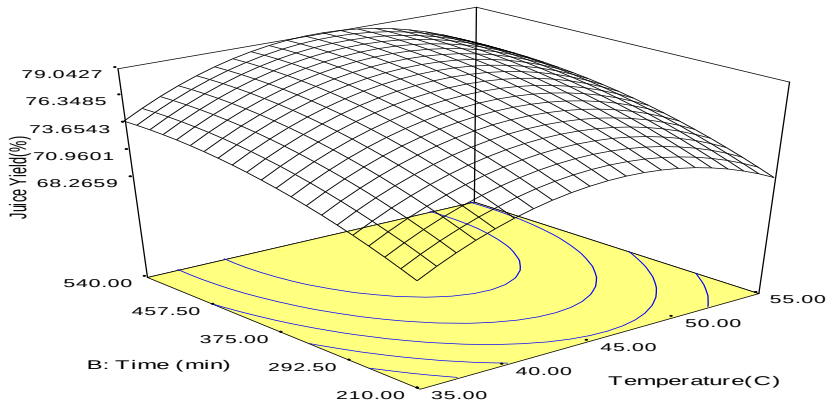
Table 4. Regression coefficients of predicted quadratic polynomial models for the responses for the model.

Cooficients	Juice Yield	Viscosity	Clarity
Intercept	+77.80 [a]	+1.55 [a]	+14.43 [a]
Linear			
b_1	+0.71 [b]	+0.036 [b]	+0.49 [b]
b_2	+2.80 [a]	-0.024	+0.84 [b]
b_3	+3.11 [a]	-0.14 [a]	+1.52 [a]
Quadratic			
b_1^2	-4.39 [a]	+0.21 [a]	-1.49 [a]
b_2^2	-1.60 [a]	+0.13 [a]	-1.41 [a]
b_3^2	-2.56 [a]	+0.10 [a]	-1.32 [a]
Crossproduct			
b_{12}	-0.025	-8.750E-003	-0.061
b_{13}	+0.37	-3.750E-003	+0.061
b_{23}	-1.98 [b]	+0.019	+0.59 [b]
R^2 [d]	0.9824	0.9786	0.9769
Adj. R^2 [e]	0.9665	0.9594	0.9562
CV [f]	1.77	3.15	5.56
F-value	61.92	50.88	47.04

Statistically significant at [a] $P< 0.001$, [b] $P< 0.05$, and [c] $P< 0.10$; [d] Coefficient of multiple determination; [e]AdjustedR^2; [f] Coefficient of variance.

Table 5. Optimization of process variables with respect to juice yield, viscosity and juice clarity.

		Optimum value (In the range)	Optimum value (Targeted)		
Variables	Temperature (°C)	45.24	45		
	Time (min)	462.69	463		
	Conc. of crude enzyme (ml/50g of pulp)	0.12	0.12		
			Predicted Value	*Experimental value*	*Deviation (%)*
Responses	Juice Yield (%)		79.25	78.00	1.60
	Viscosity (cps)		1.33	1.29	3.09
	Juice Clarity (%T)		2.15	2.12	1.30
	Desirability		0.988		



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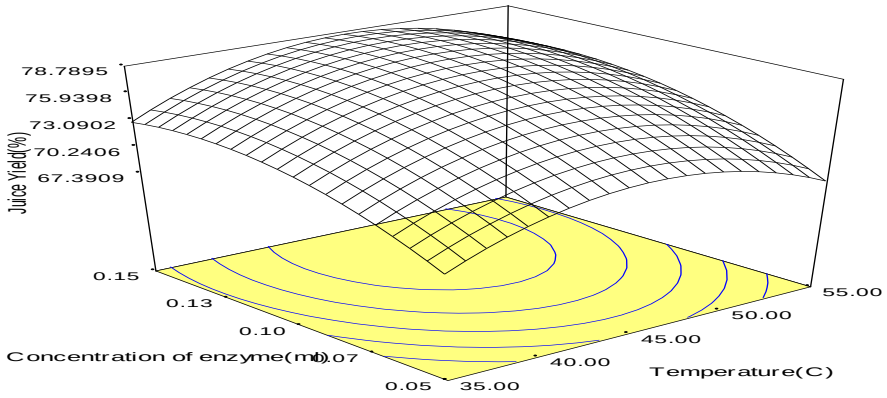
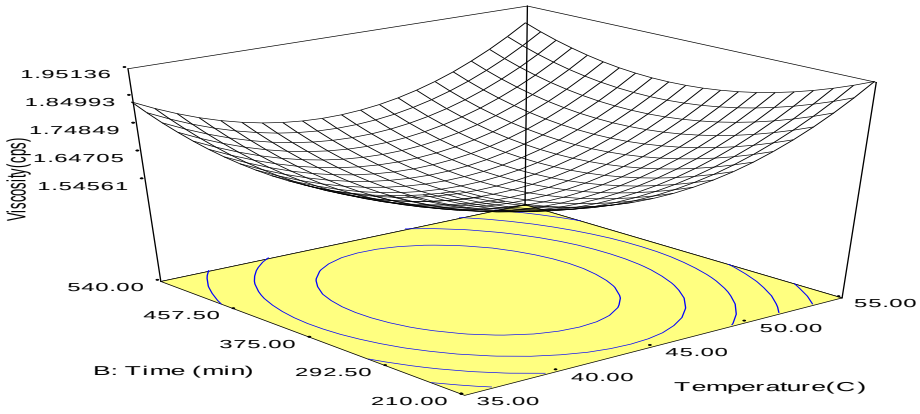


(b)

Fig. 1 Response surfaces of juice yield as a function of (a) time and temperature (b) concentration of crude enzyme and temperature.



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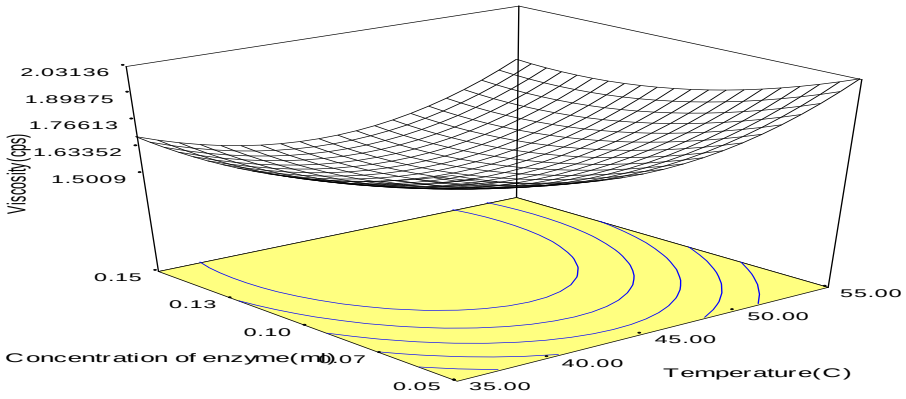
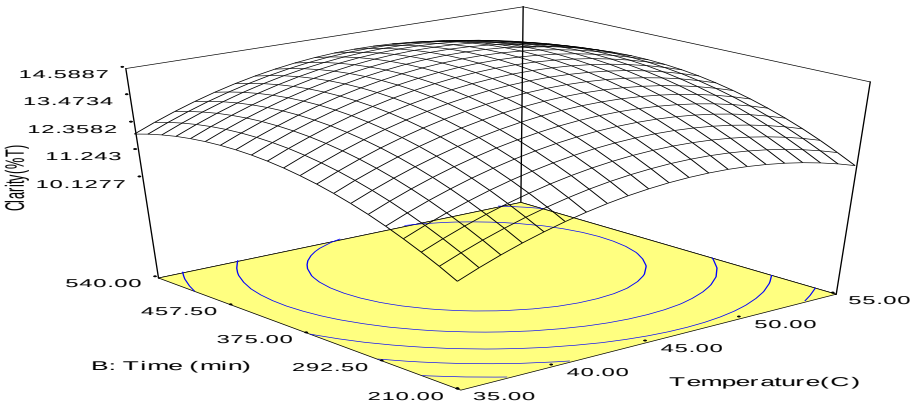
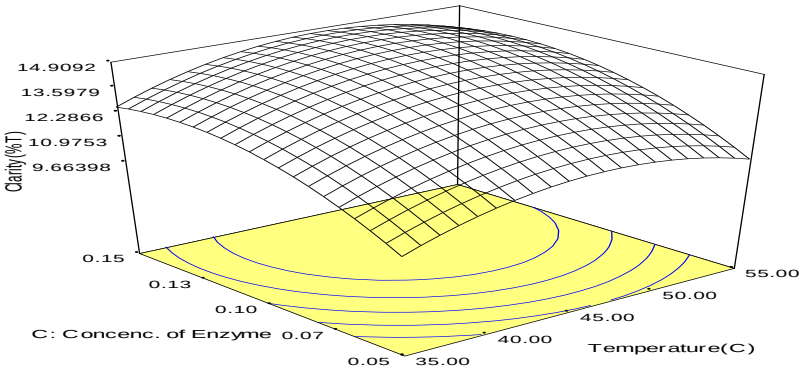


(b)

Fig. 2 Response surfaces of viscosity of juice as a function of (a) time and temperature (b) concentration of crude enzyme and temperature.



(a)



(b)

Fig. 3 Response surfaces of clarity of juice as a function of (a) time and temperature (b) concentration of crude enzyme and temperature.