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OPTIMIZATION OF ENZYMATIC HYDROLYSIS CONDITIONS FOR ENHANCED JUICE RECOVERY WITH OPTIMUM QUALITY FROM ALU BUKHARA (*PRUNUS DOMESTICA L.*) FRUIT

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Abstract: *The effect of incubation temperature (35–55 °C), incubation time (210-540 min), cellulase (6 –15 mg/50g plum pulp), and pectinase (1-2.5 mg/50g pulp) concentration on juice yield, viscosity and clarity of juice was studied. A central composite rotatable design was used to establish the optimum conditions for enzymatic hydrolysis of pulp to obtain maximum juice yield, clarity and minimum viscosity. Significant regression model describing the changes of juice yield, viscosity and clarity of juice with respect to hydrolysis parameters were established with the coefficient of determination, R^2=0.9796, 0.9687 and 0.9746 respectively. The concentration of pectinase enzyme was the most significant variable affecting the juice yield whereas viscosity and clarity of juice were most significantly affected by the concentration of cellulase and pectinase respectively. The recommended enzymatic treatment conditions were: incubation time 525 min, incubation temperature 45°C, celllulase concentration 15 mg/50g pulp and pectinase concentration 2.50 mg/50g pulp.*

Keywords: *Alu Bukhara juice; Enzymatic clarification; Pectinase; Cellulase; Statistical design*

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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 ideal weight and balanced nutrition. It contains vitamin A, B & C and minerals like calcium, magnesium, potassium and iron 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 [12]. Neochlorogenic and chlorogenic acid, two dominant phenolic compounds in prunes, were antioxidants toward isolated human LDL [6]. Consuming peaches, plums and nectarines is positively associated with nutrient intake, improves anthropometric measurements and reduced risk of hypertension [2]. 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. High amounts of polyphenols and total acid provides the health and economic requirements for the production of plum nectars.

Generally, three methods of juice extraction are employed viz, cold, hot, and enzymatic methods [20]. The use of fungal enzyme in fruit juice extraction had shown significant increase in juice recovery as compared to cold and hot extraction methods [8]. 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 [16]. 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 [1]. These parameters need to be optimized for maximal juice recovery. Therefore the

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

MATERIALS AND METHODS

2.1 Materials

Fully ripe fresh alu bukhara's (*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 Enzyme Source

Commercial enzymes pectinase and cellulase (Fluka chemicals, India) from the source organism *Aspergillus Niger* with activity 1.64 and 0.3 units/mg respectively were used for enzymatic treatment of fruit pulp.

2.3 Preliminary experiments with different concentration of commercial enzymes

Preliminary experiments were performed for the selection of the ranges and levels for the concentration of cellulase and pectinase. Different concentrations of the pectinase and cellulase were used individually (Table 1) for the treatment of alu bukhara pulp to improve the yield and the quality of the juice. The responses observed in the experiments were juice yield, viscosity and clarity of the juice (Table 1).

2.4 Selection of relevant variables and experimental ranges

The initial step was the selection of relevant factors in enzyme production and the experimental ranges for the independent variables. Four independent variables were selected for the model. The experimental ranges for the independent variables were selected as temperature in the range of 35-55^{o}C and time in range of 210 - 540 min with respect to the reported literature [10]. The ranges and levels for the concentration of cellulase (6-10mg/50g of pulp) and pectinase (1-2.5mg/50g of pulp) were selected based on the preliminary experiments (Table 1.)

2.5 Experimental Design and Statistical Analysis

Response surface methodology (RSM) was adopted in the experimental design. A five-level four-factor central composite rotatable design (CCRD) was employed. The independent variables were the temperature of enzyme treatment (X_1), time (X_2), concentration of

cellulase (X_3) and concentration of pectinase (X_4) with their levels (ranges) as given in the Table 2. The pulp, 50g was used and its pH was kept at its natural value (4.0–5.2) and was excluded from the RSM experimental design as the pH range is optimal for the exogenous pectinases [7] and cellulases. A total of 30 experiments were conducted .The four independent variables were coded as -2 (lowest level)−1, 0, +1 (middle level), and +2 (highest level). The experimental design matrix in coded form and at the actual level of variables is given in Table 2.

The response function (y) was related to the coded variables by a second degree polynomial equation (Eq. 1) as given below:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 ++ b_4 x_4 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{14} x_1 x_4 + b_{23} x_2 x_3 + b_{24} x_2 x_4 + b_{34} x_3 x_4 + b_{11} x^2_1 + b_{22} x^2_2 + b_{33} x^2_3 + b_{44} x^2_4 \quad \text{.....(1)}$$

The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2, b_3, b_4 (linear effects); b_{12}, b_{13},b_{14}, b_{23}, b_{24}, b_{34} (interaction effects); b_{11}, b_{22}, b_{33}, b_{44} (quadratic effects); and ε (random error). The statistical analysis of the data and three-dimensional plotting were performed using Design Expert software trial version '6.0.10' (Trial version; STAT-EASE Inc., Minneapolis, MN, USA).

2.6 Enzymatic treatment and juice yield

For each experiment, 50 g of pulp was subjected to different enzyme treatment conditions, as given in Table 2. The temperature of the enzymatic treatment combinations 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 suspension was filtered through six folded cheese cloth and the extract was heated at 90 °C for 5 min to inactivate the enzyme [17] using the same water bath. The extract thus collected was considered as clear juice. The juice yield was then calculated using the following expression:

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

2.7 Clarity

Juice clarity was measured according to the methods of Krop and Pilnik [13] and Ough and Crowell [15]. The juice was shaken and 10 ml portion of juice was centrifuged at 3000 rpm for 10 minutes to remove pulp or coarse cloud particles. The clarity of the juice obtained was determined by measuring the transmittance at a wavelength of 570 nm using Double

Beam 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.

2.8 Viscosity

Clean and dried Ostwald capillary viscometer was used for the measurement of viscosity. Double distilled water was used as a reference. Time required to flow through the capillary section of the Oswald viscometer was noted using a stopwatch for the reference and the sample at 20 ± 2°C [18].

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.

RESULTS AND DISCUSSION

3.1 Juice yield

The variations in juice yield due to enzymatic hydrolysis are shown in Table 4 along with the value of untreated sample. The results indicate that juice yield of untreated plum was 59% while it ranged from 74.1 to 85.5% in enzymatically treated sample depending on the experimental conditions (Table 3). The maximum juice yield (85.5%) was obtained at incubation temperature 45 °C, incubation time 375 min, cellulase concentration 10.5 mg/50g and pectinase concentration 3.25 mg/50g. This showed that enzymatic treatment enhanced juice yield by a maximum of 21.4%. The increase in juice recovery in enzymatically hydrolyzed alu bukhara samples can be attributed to the action of the pectinase and cellulase. Response surface analysis of the juice yield as a function of enzymatic hydrolysis process variables was developed by using multiple regression technique. The coefficient of determination, (R^2) was 0.9796 whereas F-value for the model was 54.14 (Table 5.) which implies the significance of model to predict juice yield at different designed conditions ($P< 0.0001$). The Pred R^2=0.9079 is in reasonable agreement with the Adj R^2=0.9606 (Table 4.). The derived model for juice recovery was obtained as:

$Y_1 = +84.47 - 0.31x_1 + 0.70x_2 + 1.51x_3 + 2.09x_4 + 0.027x_1x_2 + 0.027x_1x_3 + 0.20x_1x_4 - 0.38x_2x_3 + 0.53x_2x_4 - 0.67x_3x_4 - 1.49x_1^2 - 1.64x_2^2 - 0.66x_3^2 - 0.67x_4^2$ (Eqn.2)

Where, Y_1 is the juice yield (%), X_1, X_2, X_3 and X_4 are coded variables, the temperature, time concentration of cellulose and pectinase respectively. Further statistical analysis (Table 5) was then performed. This analysis is a joint test on all the variables involving one particular factor. Results showed that all the variables had a significant overall effect on the juice yield. The time, concentration of pectinase and cellulase (X_3) had equally significant effect.

The response surface curves for juice yield are shown in Figures. 1a-c. Each figure demonstrates the effect of two factors while the other two factors were fixed at their middle level. Figure 1a is the response surface curve for variation in the juice yield as function of incubation temperature (X_1) and incubation time (X_2), keeping the concentration of cellulose (X_3) and concentration of pectinase (X_4) at middle level 1.75mg/50g pulp and 10.50mg/50g of pulp respectively. The figure indicates that the juice yield increased with the increase in both time, 412 min and temperature, 44^{o}C. With further increase in temperature and incubation time the juice yield decreased slowly. The decrease in juice yield with increasing temperature beyond 44^{o}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 Kaur *et al*[10], who reported that the maximum juice yield from guava is obtained by pectinolytic enzyme treatment of pulp at 43.3^{o}C temperature for 447min of time.

Fig. 1b depicts the interactive effect of concentration of pectinase (X_4) and incubation temperature (X_1) to juice yield. Fig. shows that the juice yield increased with increase in temperature and concentration of pectinase upto 44 $^\circ$C of temperature and 2.50 mg (maximum concentration) of pectinase. The juice yield decreased slowly beyond 46 $^\circ$C of temperature which may be due to decrease in enzyme activity. The increase in juice yield with increasing pectinase concentration is also supported by Pilnic and Voragen [16] who reported that pectinases degrade pectic substances leading to increase in juice yield.

Figure 1c, reveals the effect of incubation time (X_2) and cellulase concentration (X_3) on juice yield. It was evident that juice yield increased with increase in time and cellulase concentration up to 340 min of incubation time and maximum concentration (15mg/50g pulp) of cellulase. The maximum production of juice under these conditions was 85%. With

further increase in the incubation time the juice yield decreased slowly. Shah [19] observed that the juice yield of litchi increased when the pulp was treated with cellulase enzyme.

3.2 Viscosity

The use of enzymes leads to the drop of fruit juice viscosity as well as improving pressibility of the pulp, disintegrating the jelly structure and making it easier to obtained the fruit juices. The variation in the viscosity of the juice under enzymatic treatment along with untreated sample is given in Table 3 where as the regression co-effcients and significance levels of the terms are given in Table 5. The results indicate that viscosity of the juice of untreated plum was 1.75 cps while it ranged from 1.47 to 1.13cps in enzymatically treated sample depending on the experimental conditions (Table 4). The minimum viscosity (1.13) was obtained at incubation temperature 45°C, incubation time 375min, cellulase concentration 10.5mg/50g and pectinase concentration 3.25mg/50g. This showed that enzymatic treatment decreased the viscosity. The decrease in juice viscosity in enzymatically hydrolyzed alu bukhara samples can be attributed to the action of the pectinases and cellulases. Response surface analysis of viscosity as a function of enzymatic hydrolysis process variables was developed by using multiple regression technique. The coefficient of determination, (R^2) was 0.9687 for the regressed model predicting the viscosity of the juice. The F-value for the model was 33.13 (Table 5.) which implies the model was significant to predict viscosity at different designed conditions ($P< 0.0001$). All the variables had a significant overall effect on the juice yield. The concentration of cellulase (X_3) and concentration of pectinase (X_2) had the most significant effect.

The response surface curves were plotted to explain the interaction of the variables. The response surface curves for viscosity of the juice are shown in Figures 2a-c. Figure 2a is the response surface curve for variation in the viscosity of the juice as function of incubation temperature (X_1) and time (X_2), keeping the concentration of cellulose (X_3) and concentration of pectinase (X_4) at middle level. It is clear from the figure that with increase in temperature and time, the viscosity decreased up to 44°C of temperature and 540min (maximum value) of time. With further increase in temperature may be due to inactivation of enzyme at higher temperature The findings are in accordance with Lee *et al*[14] who reported that the viscosity of the banana juice decreases with increase in temperature of the enzymatic treatment reaction up to 42°C. With further increase in temperature over

44°C the viscosity of juice increased. The increase in viscosity with increasing temperature may be due to inactivation of enzyme at higher temperature. 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 of incubation temperature (X_1) and pectinase concentration (X_4). The figure shows that the viscosity decreased with increase in concentration of pectinase and incubation time. The viscosity of juice decreased up to maximum concentration (2.5mg/50g of pulp) of pectinase and 44°C of incubation temperature. The minimum viscosity of juice was under these conditions was 1.17cps. The juice viscosity increased with further increase in temperature. Karangwa *et al*[9] reported the increase in viscosity of the blended carrot-orange juice with increase in temperature beyond 50°C. Lee *et al*[14] observed that the viscosity of the juice decreases with increase in enzyme concentration up to its maximum value (0.1%).

Figure 2c, reveals the effect of incubation time (X_2) and cellulase concentration (X_3) on viscosity. It was evident that with increase in concentration of cellulase and incubation time, viscosity of the juice decreased and the minimum juice viscosity was 1.17cps at the maximum concentration of cellulase, 15mg/50g of pulp and incubation time, 540 min.

3.3 Clarity of juice

The variation in juice clarity due to enzymatic hydrolysis is shown in Table 3 along with untreated samples. The results indicate that clarity of the juice from untreated alu bukhara pulp was 0.5% while it ranged from 1.0 to 17.9% in enzymatically treated sample depending on the experimental conditions (Table 3). The maximum juice clarity (17.9%) was obtained at the incubation temperature 45°C, incubation time 375min, cellulase concentration 10.5mg/50g pulp and pectinase concentration 3.25 mg/50g pulp. This showed that enzymatic treatment enhanced juice clarity by a maximum of 1.6 %. Response surface analysis of juice clarity as a function of enzymatic hydrolysis process variables was developed by using multiple regression technique. All the variables had a significant overall effect on the juice clarity (Table 5). The temperature (X_1) had the most significant effect.

The response surface curves for juice clarity are shown in Figures. 3a-c. Each figure demonstrates the effect of two factors while the other two factors were fixed at their

middle level. Figure 3a is the response surface curve for variation in the clarity of juice as function of incubation temperature (X_1) and incubation time (X_2), keeping the other two at their middle level It was evident from the figure that, clarity of juice increased with the increase in both time and temperature up to 540 min (maximum value) of time and 46^{o}C temperature respectively. With further increase in temperature, the clarity of juice decreased. Karangwa *et al*[9] observed that the clarity of the blended carrot-orange juice decreased with increase in temperature beyond 50^{o}C.

Figure 3b, presents the interaction effect of incubation temperature (X_1) and pectinase concentration (X_4) to clarity of juice. It was observed from the figure, clarity of juice increased with the increase in both concentration of pectinase and incubation temperature up to 2.50mg/50g pulp pectinase concentration and 45^{o}C temperature. 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 [5] With further increase in the incubation temperature the clarity of juice decreased.

The effect of incubation time (X_2) and cellulase concentration (X_3) on clarity of juice is shown in the figure 3c. It was observed that clarity of juice increased with increase in time and cellulase concentration up to maximum concentration (15mg/50g pulp) of cellulase and maximum incubation time (540 min). The maximum clarity of juice under these conditions was 12.3. The time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature [11].

3.4 Optimization and verification of process variables

The main criterion for constraints optimization was maximum possible juice yield and clarity and minimum viscosity of juice. Under the constraints. the optimum treatment conditions were found to be at 44.69°C, incubation temperature; 524.62min, incubation time, 15mg/50g pulp; concentration of cellulase and 2.50mg/50g pulp; concentration of pectinase (Table 6). But in practice, it is difficult to maintain the recommended conditions during processing and some deviation is expected. Therefore, optimum conditions were targeted as temperature 45^{o}C, time 525min, concentration of cellulase 15mg/50g pulp and concentration of pectinase 2.50mg/50g pulp. Under the optimum conditions (target constraints), experiments were conducted to check the variation in juice yield, viscosity and clarity of juice. The experimental values of different responses were very close to the

predicted values (Table 6) with a desirability of 0.960. This implied that there was a high fit degree between the observed and predicted values from the regression model.

3.5 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 Incubation temperature, time, concentration of pectinase and cellulase was established. The concentration of pectinase enzyme was the most significant variable affecting the juice yield whereas viscosity and clarity of juice were most significantly affected by the concentration of cellulase and pectinase respectively. The recommended enzymatic treatment conditions were: incubation time 525 min, incubation temperature 45°C, celllulase concentration 15mg/50g pulp and pectinase concentration 2.50mg/50g pulp.

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Table 1. Preliminary experiments with different concentration of commercial enzymes

Sr. No.	Conc. of Cellulase (mg/50g pulp)	Juice Yield (%)	Viscosity (cps)	Clarity (%T)	Conc. of Pectinase (mg/50g pulp)	Juice Yield (%)	Viscosity (cps)	Clarity (%T)
1.	3	74	1.82	9.5	0.5	76	1.72	10.6
2.	6	75.5	1.88	9.7	1.0	76.3	1.64	11.2
3.	9	77.3	1.76	10.2	1.5	78	1.63	12.4
4.	12	79	1.79	11	2.0	81.4	1.48	13.7
5.	15	80	1.58	11.1	2.5	81.3	1.44	13.4
6.	18	80.7	1.60	11.6	3.0	81.7	1.51	14
7.	21	80.4	1.53	10.8	3.5	82	1.42	13.2

Table 2. Experimental range and levels of the independent variables

Variables	Range and levels				
	-2	-1	0	1	2
Temperature (X_1, ^oC)	25	35	45	55	65
Time (X_2,min)	45	210	375	540	705
Concentration of Cellulase (X_3, mg)	1.50	6.0	10.5	15.0	19.5
Concentration of Pectinase (X_4, mg)	0.25	1.00	1.75	2.50	3.25

Table 3. The central composite rotatable experimental design employed for enzymatic hydrolysis pretreatment of alu Bukhara pulp

Exp. No.	Coded Variables				Uncoded Variables				Responces		
	X_1	X_2	X_3	X_4	Temp. (^oC)	Time (min.)	Conc. of Cellulase (mg)	Conc. of Pectinase (mg)	Juice Yield (%)	Viscosity (cps)	Clarity (%T)
1.	-1	-1	-1	-1	35	210	6	1	75.9	1.43	2
2.	1	-1	-1	-1	55	210	6	1	74.1	1.44	1.8
3.	-1	1	-1	-1	35	540	6	1	76.7	1.33	1.5
4.	1	1	-1	-1	55	540	6	1	75.42	1.35	1.9
5.	-1	-1	1	-1	35	210	15	1	81	1.27	5
6.	1	-1	1	-1	55	210	15	1	80.4	1.29	4.3
7.	-1	1	1	-1	35	540	15	1	79.91	1.23	9.1
8.	1	1	1	-1	55	540	15	1	79.1	1.28	10.2
9.	-1	-1	-1	1	35	210	6	2.5	80.2	1.25	8
10.	1	-1	-1	1	55	210	6	2.5	79.94	1.25	6.3
11.	-1	1	-1	1	35	540	6	2.5	82.4	1.21	8.1
12.	1	1	-1	1	55	540	6	2.5	82.6	1.25	9
13.	-1	-1	1	1	35	210	15	2.5	82.6	1.16	10.4
14.	1	-1	1	1	55	210	15	2.5	82.1	1.19	9.1
15.	-1	1	1	1	35	540	15	2.5	84.4	1.15	13.12
16.	1	1	1	1	55	540	15	2.5	83.6	1.18	12.6
17.	-2	0	0	0	25	375	10.5	1.75	78.87	1.47	1
18.	2	0	0	0	65	375	10.5	1.75	78.1	1.46	1.2
19.	0	-2	0	0	45	45	10.5	1.75	75.62	1.29	3.3
20.	0	2	0	0	45	705	10.5	1.75	80.12	1.18	11.6
21.	0	0	-2	0	45	375	1.5	1.75	79.15	1.29	4.9
22.	0	0	2	0	45	375	19.5	1.75	84.4	1.14	13.9
23.	0	0	0	-2	45	375	10.5	0.25	78.02	1.37	6.2
24.	0	0	0	2	45	375	10.5	3.25	85.5	1.13	17.9
25.	0	0	0	0	45	375	10.5	1.75	84.92	1.22	8.1
26.	0	0	0	0	45	375	10.5	1.75	84.56	1.26	6.81
27.	0	0	0	0	45	375	10.5	1.75	84.62	1.22	8.24
28.	0	0	0	0	45	375	10.5	1.75	83.4	1.24	7.63
29.	0	0	0	0	45	375	10.5	1.75	84.2	1.23	6.51
30.	0	0	0	0	45	375	10.5	1.75	85.1	1.22	7.2

Table 4. The range of different parameters (Juice Yield, Apparent viscosity and Clarity) of juice obtained from untreated and enzyme treated alu bukhara pulp.

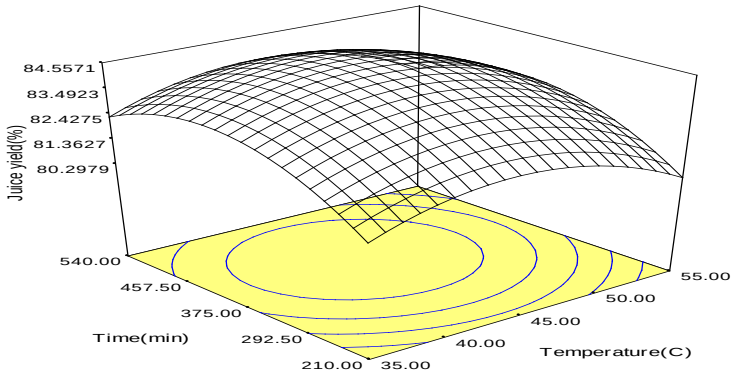
Parameters	Units	Untreated	Enzyme treated
Juice Yield	% w/v	59	74.1 – 85.5
Juice apparent Viscosity	Cps	1.75	1.46 - 1.13
Juice Clarity	% T	0.5	1 - 17.9

Table 5. Analysis of variance table (Partial sum of squares) for response surface quadratic models for juice yield, viscosity and clarity of the juice.

Source	Juice yield			Viscosity			Clarity		
	Sum of Squares	F- Value	p-value Prob > F	Sum of Squares	F- Value	p-value Prob > F	Sum of Squares	F- Value	p-value Prob > F
Model	310.74	51.54	< 0.0001	0.2455	33.13	< 0.0001	490.78	41.18	< 0.0001
A	2.28	5.28	0.0363	0.0013	2.54	0.1312	0.10935	0.12	0.7250
B	11.89	27.60	< 0.0001	0.0112	21.28	0.0003	51.685	60.71	< 0.0001
C	55.06	127.85	< 0.0001	0.0468	88.42	< 0.0001	118.01	138.64	< 0.0001
D	105.29	244.52	< 0.0001	0.0888	167.75	< 0.0001	171.842	201.87	< 0.0001
A^2	60.65	140.84	< 0.0001	0.0804	152.00	< 0.0001	75.373	88.54	< 0.0001
B^2	73.84	171.47	< 0.0001	0.0003	0.5756	0.4598	0.1352	0.15	0.6959
C^2	12.11	28.12	< 0.0001	0.0019	3.60	0.0773	4.7762	5.61	0.0317
D^2	12.25	28.44	< 0.0001	4.76E-06	0.0089	0.9257	31.980	37.57	< 0.0001
AB	0.014	0.032	0.8603	0.0004	0.7555	0.3984	2.0880	2.45	0.1382
AC	0.012	0.027	0.8721	0.0002	0.4249	0.5243	0.0420	0.049	0.8272
AD	0.61	1.42	0.2516	0	0	1.0000	0.6480	0.76	0.3967
BC	2.30	5.35	0.0354	0.0016	3.0220	0.1026	11.937	14.02	0.0020
BD	4.44	10.31	0.0058	0.0020	3.8247	0.0694	0.0210	0.024	0.8772
CD	7.20	16.31	0.0010	0.0025	4.72	0.0462	3.5910	4.21	0.0578
Residual	6.46			0.0079			12.768		
Lack of Fit	4.61	1.25	0.4266	0.0066	2.59	0.1522	10.341	2.14	0.2090
Pure Error	1.85			0.0012			2.42735		
Cor Total	317.20			0.2535			503.55		
R-Square	0.9796			0.9687			0.9746		
Adj R-Square	0.9606			0.9394			0.9510		
Pred R-Square	0.9079			0.8414			0.8748		
Adeq Precision	124.509			27.716			25.194		
PRESS	29.22			0.040			63.06		

Table 6. Optimization of process variables with respect to juice yield, viscosity and juice clarity.

		Optimum value (In the range)	Optimum value (Targeted)		
Variables	Temperature (°C)	44.69	45.00		
	Time (min)	524.62	525.00		
	Conc. of cellulase (mg/50g pulp)	15.00	15.00		
	Conc. of pectinase (mg/50g pulp)	2.50	2.50		
			Predicted Value	Experimental value	Deviation (%)
Responses	Juice Yield (%)		85.50	83.70	2.15
	Viscosity (cps)		1.13	1.12	0.89
	Juice Clarity (%T)		15.35	14.65	4.77
	Desirability		0.960		



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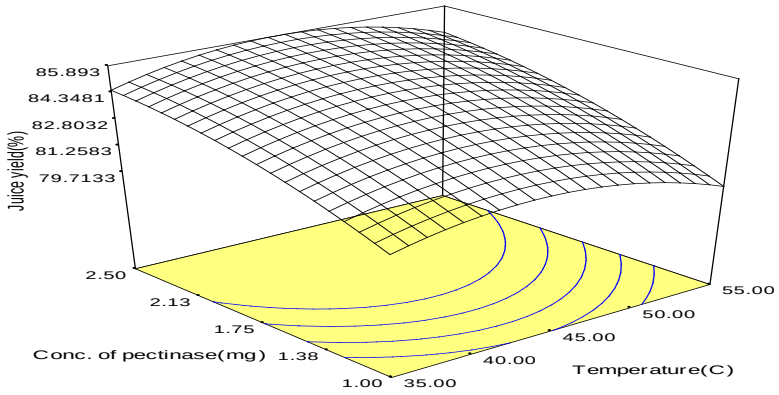

(b)

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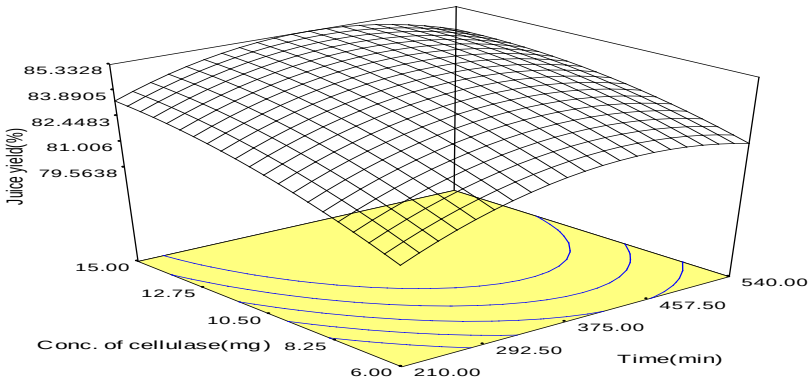
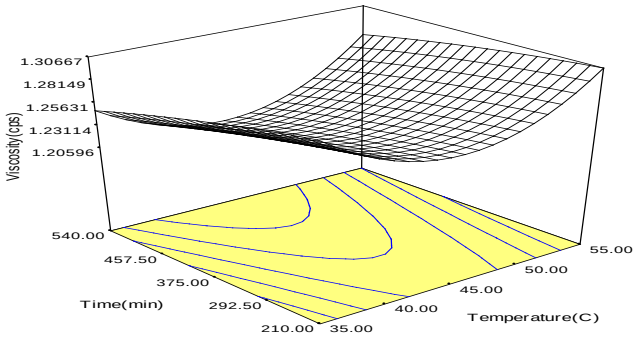


(c)

Fig. 1 Response surfaces of juice yield as a function of (a) temperature and time (b) temperature and concentration of pectinase (c) time and concentration of cellulase



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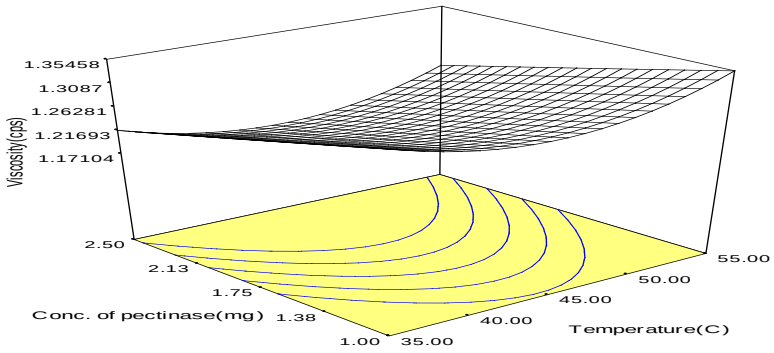
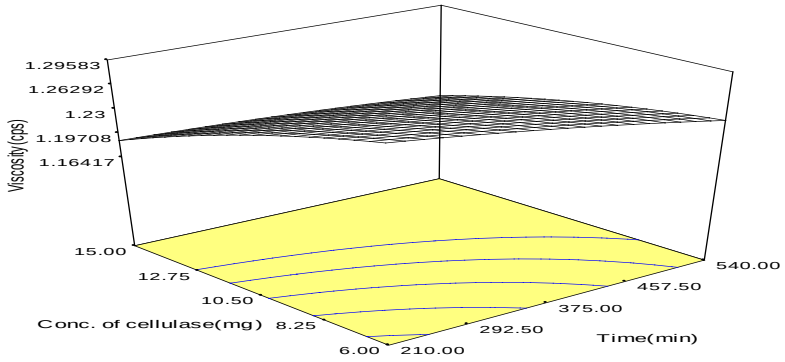
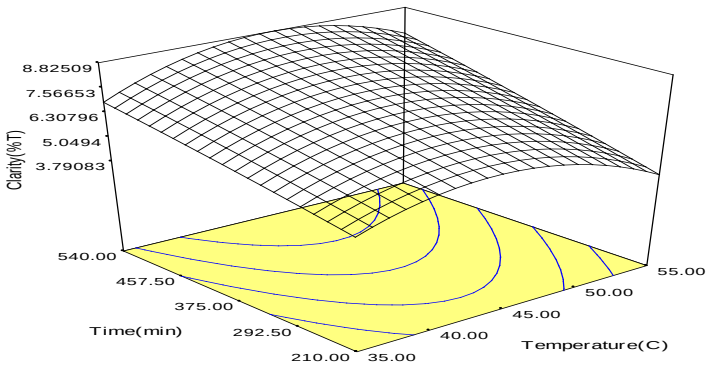


(b)



(c)

Fig. 2 Response surfaces of viscosity of the juice as a function of (a) temperature and time (b) temperature and concentration of pectinase (c) time and concentration of cellulase



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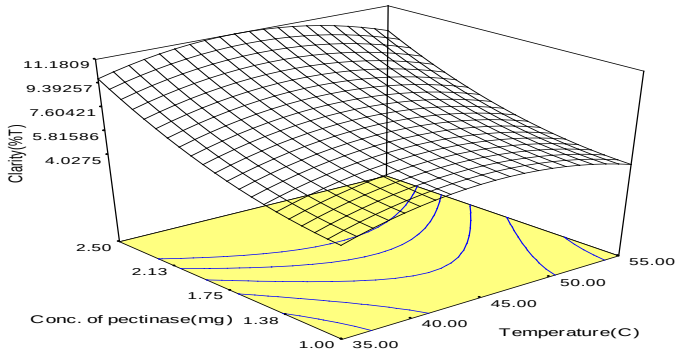

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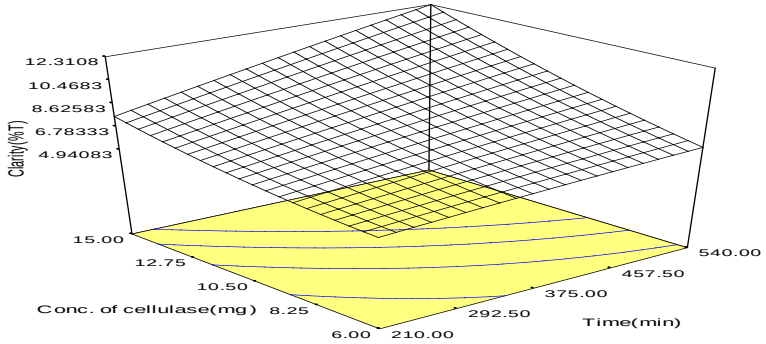

(c)

Fig. 3 Response surfaces of clarity of the juice as a function of (a) temperature and time (b) temperature and concentration of pectinase (c) time and concentration of cellulase