



American Journal of **Food Technology**

ISSN 1557-4571



Academic
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Effect of Enzymatic Hydrolysis on the Juice Yield from Bael Fruit (*Aegle marmelos* Correa) Pulp

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ABSTRACT

The effect of incubation temperature (28.18-61.82°C), incubation time (97.5-652.5 min) and pectinase concentration (0.64-7.36 mg/25 g bael pulp) on juice yield, viscosity and clarity of juice were studied. A central composite rotatable design was used to optimize the conditions for enzymatic hydrolysis of bael to maximize juice yield and clarity and to minimize 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, $R^2 = 0.9750$, 0.9507 and 0.9516 , respectively and the range of different parameters (juice yield, viscosity and clarity) of juice obtained from enzyme treated bael pulp was 72.5-86.6%, 1.34-1.66 (cps) and 17.7-28.9% T, respectively. The recommended enzymatic treatment conditions were: Incubation time 425 min, incubation temperature 47°C and pectinase concentration 5.0 mg/25 g bael pulp and the juice yield, viscosity and clarity under these conditions were 84.5%, 1.35 cps and 22.43% T, respectively.

Key words: Bael juice, enzymatic hydrolysis, optimization, pectinase

INTRODUCTION

The bael fruit (*Aegle marmelos* Correa, family: Rutaceae), occupies an important place among the various fruits. It is known by different names viz. Bael, Bel, Bengal Quince, Bil, Bilva, Bilpatre, Shul, Shaiphal and Vilvum etc. The main cultivated varieties are Mirzapuri, Kagzi, Gonda, Kagzi Banarsi, Kagzi Etawah, Narendra Bael-1, Narendra Bael-2, Pant Aparna and Pant Sujata. (Rakesh *et al.*, 2005). It grows throughout the Indian Peninsula as well as in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand and most of the southeastern Asian countries. It is very hardy subtropical, deciduous tree that can thrive well in various soil climate conditions (from swampy to dry soils) and can tolerate alkaline soil and is not injured by low temperature as -7°C (Rakesh *et al.*, 2005).

Bael fruit has been attributed with various nutritional and therapeutic properties such as in the cure of chronic diarrhea and certain other gastrointestinal disorders. The marmelosin ($C_{13}H_{12}O_3$) content is found in the bael fruit which is known as panacea of stomach ailments (Singh and Nath, 2004). The unripe fruits are astringent, digestive, stomachic and are prescribed in case of diarrhea and dysentery (Pande *et al.*, 1986). The ripe fruit is sweet, aromatic, nutritious and very palatable being highly esteemed and eaten by all classes of people. The fruit has excellent aroma which is not

destroyed even during processing. Bael Fruit because of its hard shell, mucilaginous texture and numerous seeds, it is not popular as fresh fruit. Although excellent flavor, nutritive and therapeutic value of bael fruits shows potential for processing into value added products.

Bael is usually processed into products like preserves, refreshing beverages, powder, leather, squash, nectars, toffee, jam, syrup. Further bael fruit flavor is entirely unknown in internal and export markets. Therefore, the value added products from bael can attract both internal and export markets. Although, few workers have reported some work on the development and evaluation of bael products, yet, there is a paucity of literature on processing technology of bael fruit pulp. Despite vast potential of either pure or mixtures with other juices, bael juice has not been exploited for its commercialization. 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 (Joshi *et al.*, 1991). The pectinase enzymes assist in pectin hydrolysis, which cause a reduction in pulp viscosity and a significant increase in juice yield (Pilnik and Voragen, 1993).

The enzymatic hydrolysis of pectic substances depends on several processing variables such as type of enzyme, hydrolysis time, enzyme concentration, incubation temperature and pH (Baumann, 1981). These parameters need to be optimized for maximum recovery of juice. Therefore, the present study was undertaken to optimize the hydrolysis pretreatment parameters like incubation temperature, time of treatment and concentration of pectinase enzyme for the maximal juice yield from bael fruit pulp.

MATERIALS AND METHODS

Materials: Fully ripe fresh bael fruits (*Aegle marmelos* Correa) of Kagazi variety, without any visual defects, were purchased from Agricultural farm of R.B.S. College, Bichpuri, Agra (India). The bael fruits were broken by hammering and the pulp was scooped out with the help of stainless steel spoon. The scooped pulp was homogenized by blending manually. The fresh pulp so prepared was used to extract juice.

Enzyme source: Pectinase Enzyme (Fluka chemicals, India) from the source organism *Aspergillus niger* with activity 1.64 units mg⁻¹ was used for enzymatic treatment of fruit pulp.

Preliminary experiments with different concentration of commercial enzymes: Preliminary experiments were performed for the selection of the ranges and levels for the concentration of pectinase. The different concentrations of the pectinase were used (Table 1) for the treatment of bael pulp and the juice yield and its quality in the form of viscosity and clarity were observed (Table 1).

Selection of relevant variables and experimental ranges: The initial step was the selection of experimental ranges for the independent variables. The experimental ranges for the independent variables were selected as temperature in the range of 35-55°C and time in range of 210-540 min with respect to the reported literature (Kaur *et al.*, 2009). The ranges and levels for the concentration of pectinase (2-6 mg/25 g of pulp) were selected based on the preliminary experiments (Table 1).

Experimental design and statistical analysis: Response surface methodology (RSM) was adopted in the experimental design. A five-level three-factor Central Composite Rotatable Design (CCRD) was employed. The independent variables were the temperature of enzyme treatment (X₁),

time (X_2) and concentration of pectinase (X_3) (Table 2). The pulp, 25 g was used and its pH was kept at its natural value (5.0-5.5) and was excluded from the RSM experimental design as the pH range is optimal for the exogenous pectinases (Kumar *et al.*, 2011). A total of 20 experiments were conducted. The three independent variables were coded as -1.68 (lowest level) -1, 0, +1 (middle level) and +1.68 (highest level). The experimental design matrix in coded (x) form and at the actual level of variables is given in Table 3. The response function (Y) was related to the coded variables (x_i , $i = 1, 2$ and 3) by a second degree polynomial equation (Eq. 1) as given below:

Table 1: Preliminary experiments with different concentration of pectinase

Conc. of pectinase (mg/25 g pulp)	Juice yield (%)	Viscosity (cps)	Clarity (%T)
1.0	73.5	1.59	18.6
2.0	75.3	1.52	21.2
3.0	79.8	1.48	22.7
4.0	82.9	1.44	23.6
5.0	83.7	1.39	24.8
6.0	84.1	1.36	26.1
7.0	84.8	1.35	26.7

Table 2: Experimental range and levels of the independent variables

Variables	Range and levels				
	-1.68	-1	0	1	1.68
Temp. (X_1 , °C)	28.18	35.0	45.0	55.0	61.82
Time (X_2 , min)	97.05	210.0	375.0	540.0	652.50
Conc. of pectinase enzyme (X_3 , mg/25 g pulp)	0.64	2.0	4.0	6.0	7.36

Table 3: The central composite rotatable design employed for enzymatic hydrolysis pretreatment of bael

Exp. No.	Coded variables			Responses		
	X_1	X_2	X_3	% Yield	Viscosity (cps)	Clarity (%T)
1	-1	-1	-1	72.9	1.53	21.1
2	1	-1	-1	72.7	1.66	22.4
3	-1	1	-1	73.8	1.48	18.1
4	1	1	-1	75.4	1.42	22.4
5	-1	-1	1	79.9	1.34	22.4
6	1	-1	1	78.5	1.56	21.4
7	-1	1	1	82.4	1.38	20.5
8	1	1	1	82.8	1.42	22.5
9	-1.682	0	0	72.8	1.47	24.8
10	1.682	0	0	74.98	1.59	28.9
11	0	-1.682	0	75.7	1.51	20.1
12	0	1.682	0	82.6	1.43	20.0
13	0	0	-1.682	72.5	1.56	17.7
14	0	0	1.682	86.6	1.41	19.4
15	0	0	0	84.2	1.41	24.3
16	0	0	0	84.5	1.36	22.4
17	0	0	0	82.7	1.36	24.0
18	0	0	0	83.3	1.39	23.0
19	0	0	0	81.3	1.41	24.5
20	0	0	0	83.6	1.38	22.5

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

where, Y is the measured response β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are the coded value of the i th and j th independent variables. The variable $X_i X_j$ represents the first order interaction between X_i and X_j .

Enzymatic treatment and juice yield: As the bael fruit pulp has very mucilaginous texture, it is difficult to extract the juice from the pulp without water addition. Therefore, preliminary experiments were carried out to decide the amount of water to be added to the pulp. For each experiment, 25 g of pulp was added with 2.5 times (62.5 g) water and subjected to different enzyme treatment conditions. The temperature of the enzymatic treatment combinations was adjusted to the desired level by using a high precision water bath (Seco, Model 129, India). At the end of the enzyme treatment, the suspension was filtered through 6 fold cheese cloth and the extract was heated at 90°C for 5 min to inactivate the enzyme (Rai and Mishra, 2003) using the same water bath. The extract thus collected was considered as clear juice. The juice yield was then calculated using the following expression:

$$\text{Juice yield (\%)} = \frac{\text{Weight of clear juice}}{\text{Weight of sample}} \times 100$$

Clarity: Juice clarity was measured according to the methods of Krop and Pilnik (1974) and Ough and Crowell (1979). The juice was shaken and 10 ml portion of juice was centrifuged at 3000 rpm for 10 min to remove pulp coarse cloud particles. The clarity of the juice obtained was determined by measuring the transmittance at a wavelength of 590 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.

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 Ostwald viscometer was noted using a stopwatch for the reference and the sample at 20±2C (Ranganna, 1997):

$$\text{Apparent viscosity } \frac{\eta}{\eta_w} = \frac{D_s \times t_s}{D_w \times t_w}$$

Where:

D = Density

t = Time of flow

s = Sample

w = Water

RESULTS AND DISCUSSION

Juice yield: The variations in juice yield due to enzymatic hydrolysis are shown in Table 4. The results showed that the juice yield of untreated sample was 69.1% while it ranged from 72.5 to

Table 4: The range of different parameters (Juice Yield, Viscosity and Clarity) of juice obtained from untreated and enzyme treated bael pulp

Parameter	Units	Untreated	Enzyme treated
Juice yield	% w/w	69.01	72.5-86.06
Juice viscosity	Cps	01.69	01.34-1.66
Juice clarity	% T	17.04	17.7-28.09

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	Sum of Squares	F- value	Sum of squares	F- value
Model	423.88	43.30	0.14	21.43	118.31	21.85
A	1.21	1.11	0.021	28.45	13.34	22.16
B	35.45	32.36	0.020	27.68	1.15	1.92
C	201.92	184.32	0.030	41.50	2.34	3.90
A ²	156.01	142.41	0.030	40.76	18.22	30.28
B ²	29.49	26.92	8.412E-003	11.56	23.60	39.21
C ²	23.95	21.86	0.013	17.19	47.21	78.45
AB	1.62	1.48	0.017	23.51	4.50	7.48
AC	0.72	0.66	4.512E-003	6.20	2.64	4.40
BC	1.28	1.17	4.512E-003	6.20	0.61	1.01
Residual	10.96		7.278E-003		16.02	
Lack of fit	4.26	0.64	4.728E-003	1.85	1.68	0.39
Pure error	6.69		2.550E-003		4.34	
Cor total	437.89		0.15		124.33	
R-square	0.9750		0.9507		0.9516	
Adj R-square	0.9525		0.9063		0.9080	
Pred R-square	0.9041		0.7326		0.8472	
Adeq precision	18.963		16.352		19.431	
Press	42.01		0.039		19.00	

86.6% in enzymatically treated sample depending on the experimental conditions (Table 4). The maximum juice yield (86.6%) was obtained at incubation temperature 45°C, incubation time 375 min and pectinase concentration 7.36 mg/25 g (Table 3). This showed that enzymatic treatment enhanced juice yield by a maximum of 17.5%. The increase in juice recovery in enzymatically hydrolyzed bael pulp samples can be attributed to the action of the pectinase. Upon enzyme treatment, degradation of pectin leads to reduction in water holding capacity of pectin. Free water is released to the system and hence the viscosity decreases and yield increases (Kashyap *et al.*, 2001; Lee *et al.*, 2006). 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 0.9750 whereas F-value for the model was 43.30 (Table 5) which implies the significance of model to predict juice yield at different designed conditions ($p < 0.001$). The Pred $R^2 = 0.9041$ is in reasonable agreement with the Adj $R^2 = 0.9545$ (Table 5). The derived model for juice recovery was obtained as:

$$Y_1 = +83.26 + 0.30x_1 + 1.61x_2 + 3.85x_3 + 0.45x_1x_2 - 0.30x_1x_3 + 0.40x_2x_3 - 3.29x_1^2 - 1.43x_2^2 - 1.29x_3^2 \quad (2)$$

Table 6: Regression coefficients of predicted quadratic polynomial models for the responses for the model

Coefficients	Juice yield	Viscosity	Clarity
Intercept	+83.26 ^a	+1.39 ^a	+23.46 ^a
Linear			
A	+0.30	+0.39 ^a	+0.99 ^a
B	+1.61 ^b	-0.38 ^a	-0.29
C	+3.85	-0.047 ^a	+0.41 ^c
Quadratic			
A ²	-3.29 ^a	+0.045 ^a	+1.12 ^a
B ²	-1.43 ^b	+0.024 ^b	-1.28 ^a
C ²	-1.29 ^a	+0.029 ^b	-1.81 ^a
Crossproduct			
A*B	+0.45	-0.046 ^a	+0.75 ^b
A*C	-0.30	+0.024 ^b	-0.57 ^c
B*C	+0.40	+0.024 ^b	+0.28

Statistically significant at ^ap<0.001, ^bp<0.05 and ^cp<0.10

where, Y_1 is the juice yield, X_1 , X_2 and X_3 are the coded factors of incubation temperature, incubation time and concentration of pectinase enzyme, respectively. Further statistical analysis (Table 6), showed that all the variables had a significant overall effect on the juice yield.

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 pectinase enzyme (X_3) at middle level i.e., 4.0 mg/25 g of bael pulp which indicates the nonlinear behaviour as also evidenced from model. The juice yield increased with the increase in both time and temperature up to 475.78 min of time and 46°C temperature. With further increase in temperature, the yield slightly decreased but was unaffected with increase in incubation time. The decrease in juice yield with increasing temperature may be due to denaturation of protein which leads to decrease in enzyme activity at higher temperature. The results are in agreement with the findings of Kaur *et al.* (2009) 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.

Figure 1b, presents the interaction effect of incubation temperature (X_1) and pectinase concentration (X_3) to juice yield. It is clear from the Fig. 1b, that at higher temperature and enzyme concentration, the juice yield followed a linear behaviour which reflects that with increase in enzyme concentration and temperature, juice yield increased upto 5.94 mg/25 g pulp of enzyme concentration and 44.92°C temperature respectively, therefore in incubation temperature reduced the juice yield which may be due to decrease in enzyme activity. The increase in juice yield may be due to the fact that pectinases degrade pectic substances leading to increase in juice yield.

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 4 where as the regression co-efficients and significance levels of the terms are given in Table 6. The results indicate that viscosity of the juice of untreated bael was 1.69 cps while it ranged from 1.34 to 1.66 cps in enzymatically treated sample depending on the experimental conditions (Table 4). The minimum viscosity (1.34) was obtained at incubation temperature 35°C, incubation time 210 min and pectinase concentration 6.0 mg/25 g. This showed

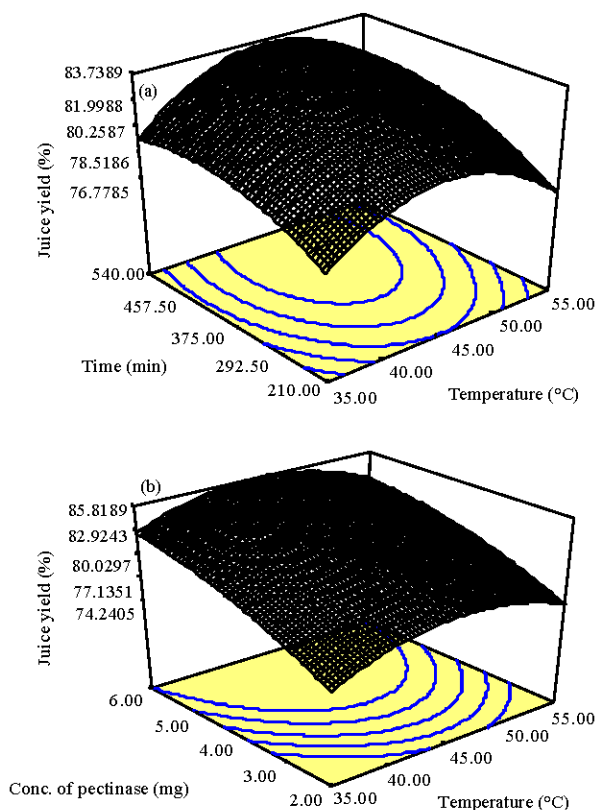


Fig. 1(a-b): Response surfaces of juice yield as a function of (a) time and temperature (b) concentration of pectinase enzyme and temperature

that enzymatic treatment decreased the viscosity. The decrease in juice viscosity in enzymatically hydrolyzed bael samples can be attributed to the action of the pectinases. The pectinase hydrolyses pectin and cause pectin-protein complexes to flocculate. The resulting juice from pectinase treatment will have a much lower amount of pectin and a lower viscosity. 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 0.9507 for the regressed model whereas, the F-value for the model was 21.43 (Table 5). Which implies the model was significant to predict viscosity at different designed conditions ($p < 0.001$). All the variables had a significant overall effect on the juice yield. The concentration of pectinase (X_3) had the most significant effect.

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable for maximum response. The response surface curves for juice yield are shown in Fig. 2a, b. Figure 2a shows the effect of incubation temperature (X_1) and incubation time (X_2) on viscosity of juice, keeping the other factor at its middle level i.e., enzyme concentration 4.0 mg/25 g of pulp. The viscosity decreased with increase in both time and temperature up to 497.86 min of time and 44.25°C temperature. With further increase in time and temperature, the viscosity of juice increased linearly. The findings are in accordance with Lee *et al.* (2006) who reported that the viscosity of the banana juice decreases with increase in temperature

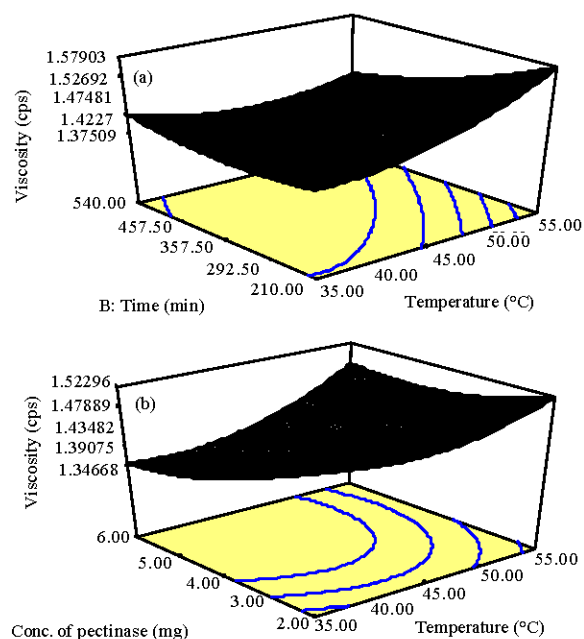


Fig. 2(a-b): Response surfaces of viscosity of juice as a function of (a) time and temperature (b) concentration of pectinase enzyme and temperature

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. 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 presents the interaction effect of incubation temperature (X_1) and pectinase concentration (X_2). The viscosity decreased with the increase in both concentration of enzyme and incubation temperature up to 5.93 mg/25 g of pulp enzyme concentration and 37.96°C temperature. With further increase in enzyme concentration, the viscosity of juice remains unaffected whereas it increased linearly with the increase in temperature up to its maximum level. Karangwa *et al.* (2010) reported increase in viscosity of the blended carrot-orange juice with increase in temperature beyond 50°C. Lee *et al.* (2006) observed that the viscosity of the juice decreases with increase in enzyme concentration up to its maximum value (0.1%).

Juice clarity: The variation in juice clarity due to enzymatic hydrolysis is shown in Table 3. The results indicate that clarity of the juice from untreated bael pulp was 17.4% while it ranged from 17.7 to 28.9% in enzymatically treated sample depending on the experimental conditions (Table 3). The maximum juice clarity (28.9%) was obtained at the incubation temperature 61.82°C, incubation time 375 min pectinase concentration 4.0 mg/25 g pulp. This showed that enzymatic treatment enhanced juice clarity by a maximum of 11.5 %. Increase in enzyme concentration may increase the rate of clarification by exposing part of the positively charged protein beneath, thus reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and eventually settled out (Sin *et al.*, 2006). 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 6). The temperature (X_1) had the most significant effect.

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable for maximum response. The response surface curves for juice yield are shown in Fig. 3a and b.

Figure 3a shows the effect of incubation temperature (X_1) and time (X_2) on juice clarity keeping the third at its middle level. The clarity of the juice increased with both incubation time and temperature up to 406.55 min and 54.86°C temperature respectively. With further increase in temperature, the clarity of juice decreased. Karangwa *et al.* (2010) observed that the clarity of the blended carrot-orange juice decreased with increase in temperature beyond 50°C.

Figure 3b reveals the effect of incubation temperature (X_1) and pectinase enzyme concentration (X_3) on the clarity of juice. It was evident that clarity of juice increased with increase in temperature and crude enzyme concentration up to 54.89°C incubation temperature and 3.79 mg (per 25 g of pulp) of pectinase concentration. The maximum clarity of juice under these conditions was 25.53%T. 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 (Demir *et al.*, 2000). With further increase in the incubation temperature the clarity of juice decreased.

Optimization and verification of process variables: The main criterion for constraints optimization was maximum juice yield and clarity and minimum viscosity. Under the constraints, the optimum treatment conditions were found to be temperature 46.57°C, time 425.21 min and

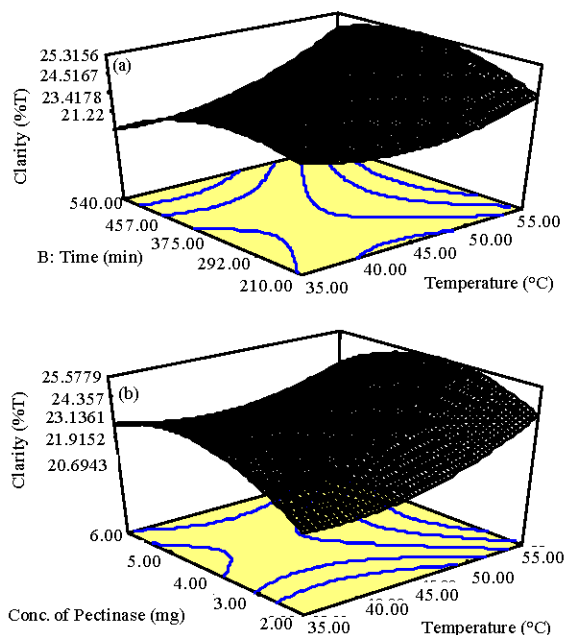


Fig. 3(a-b): Response surfaces of clarity of juice as a function of (a) time and temperature (b) concentration of pectinase enzyme and temperature

Table 7: Optimization of process variables with respect to juice yield, viscosity and juice clarity

Variables	Optimum value (In the range)	Optimum value (Targeted)	
Temperature (°C)	46.57	047.00	
Time (min)	425.21	425.00	
Conc. of pectinase (mg/25 g of pulp)	004.96	005.00	
Responses	Predicted value	Experimental value	Deviation (%)
Juice yield (%)	85.19	84.05	0.69
Viscosity (cps)	01.37	01.35	1.45
Juice clarity (%T)	23.25	22.43	3.54

concentration of pectinase enzyme 4.96 mg/25 g of pulp. 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 47°C, time 425 min and concentration of pectinase 5.0 mg/25 g of pulp. Under the optimum condition (target constraint), experiments were conducted for checking the variation in juice yield, viscosity and clarity. The experimental values of different responses under the optimum conditions of different variables are given in Table 7, which showed that the experimental results were very close to the predicted one. This implied that there was a high fit degree between the values observed in experiments and the values predicted from the regression model.

CONCLUSION

The present study concluded that bael 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 (enzyme concentration, temperature and incubation time) were established hence the response surface methodology was successfully used for the optimization of enzymatic pre-treatment conditions for the improvement of juice recovery and quality from bael. The concentration of pectinase enzyme was the most significant variable affecting the juice yield and incubation temperature was the most significant variable affecting the viscosity and clarity of the juice.

PRACTICAL APPLICATIONS

This study assesses how process variables of enzymatic treatment of bael pulp, particularly incubation temperature, incubation time and pectinase concentration affect the % yield, viscosity and clarity of the juice. The enzymatic hydrolysis of bael pulp improved the juice yield and quality, in terms of viscosity and clarity. The process is industrially applicable to improve the yield and quality of the bael juice.

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