Food Sci. Biotechnol. 21(4): 959-967 (2012)
DOI 10.1007/s10068-012-0126-x

RESEARCH ARTICLE

Comparative Effect of Crude and Commercial Enzyme on the Juice Recovery from Pineapple (*Ananas comosus*) Using Principal Component Analysis (PCA)

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Received: 14 November 2011 / Revised: 14 April 2012 / Accepted: 16 April 2012 / Published Online: 31 August 2012

Abstract In present study, the effect of crude and commercial enzyme treatment on pineapple (*Ananas comosus*) juice yield, clarity, and viscosity was studied. A central composite face centered design was used to establish the optimum conditions for enzymatic hydrolysis of pineapple using crude enzyme. The optimized crude enzyme treatment conditions were incubation temperature 47°C, incubation time 446 min, and enzyme concentration 0.14 mL/50 g of pulp. The conditions for the commercial enzyme treatment of the same variety of pineapple to improve the juice recovery and quality were also optimized. The data showed that the crude enzyme was competitive to the commercial enzymes for the improvement of juice recovery and quality from pineapple. The comparison was done under optimized conditions using principal component analysis. As viscosity was the most uncorrelated variable, hence it can be used as a variable to study variance among samples treated by crude and commercial enzymes.

Keywords: pineapple juice, crude enzyme, commercial enzyme, response surface methodology, principal component analysis

Introduction

Pineapple (*Ananas comosus*), the second most popular tropical fruit next to bananas is a good source of vitamins, minerals, fiber, and enzymes. It contains vitamin A, B_1, B_2, B_6, C, and minerals like calcium, magnesium, potassium, iron, and zinc (1). It can be eaten raw or used to make juice and other products. The extraction of pineapple juice on large scale bases includes a 2-step pressing of pineapple pulp. The residual pulp (pomace) remaining after juice extraction still contains some valuable extractable materials such as particulate, flavor, soluble solids, etc, which would improve the final quality of the juice. By adding cell wall liquefying enzymes, these valuable juice components can be extracted from the pulp.

The production of fruit and vegetable juices require methods for extraction, clarification, and stabilization. During the early 1930s, when fruit industries began to produce juice, the yields were low and many difficulties were encountered in filtering the juice to an acceptable clarity (2). Subsequently, research on industrially suitable pectinases, cellulases, and hemicellulases from food-grade microorganisms (*Aspergillus niger* and *Trichoderma* spp.), together with increased knowledge on fruit components, helped to overcome these difficulties (3).

Nowadays a combination of different pectinases (pectin lyase, pectin methylesterase, endo- and exo-polygalacturonases, pectin acetylesterase, rhamnogalacturonase, and endo- and exo-arabinases), cellulases (endoglucanases, exoglucanases, and cellobiases), and hemicellulases (endo- and exo-xylanases, galactanases, xyloglucanases, and mannanases) called as macerating enzymes are used in the extraction and clarification of fruit and vegetable juices (3,4). During the production of juice from fruits such as apples, pineapples, guava, oranges, kinnow, and pears, the whole fruits are crushed to make pulp, which is mechanically processed (pressed, filtered, and centrifuged) to yields a clear fruit juice and solid pomace.

The enzymatic process offers a number of advantages over mechanical processing of several fruit pulps. The use

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of cellulases and pectinases for the fruit processing not only facilitate easy pressing and increase in juice recovery but also improves the quality of the juices in terms of clarity, viscosity, and filterability. These enzymes not only help in softening the plant tissue but also lead to release of cell contents resulting into high juice yield. The pectinases and cellulases are enzymes which act upon the pectin and cellulose polysaccharides, respectively. These enzymes are used to remove cell walls or crude fiber to release valuable components (flavors, enzymes, polysaccharides, and other proteins) from plant cells to improve nutritional value of fruit and vegetable juices. The use of these macerating enzymes increases both yield and quality without additional capital investment.

Pineapple is commercially considered as an important fruit but the potential of the fruit is not fully tapped. The area requires wider research in terms of utilization of residue, enhanced juice yield with optimum overall acceptability. The application of commercial enzyme for the juice yield and clarification is reported (5,6). But the cost of the processing becomes a limiting factor in the application of commercial enzymes. The use of crude enzyme for the improvement of juice yield, clarity is more economical and eco-friendly, provided it is produced from generally recognized as safe (GRAS) fermentaion and should be spore and cell free. There for the present study was undertaken to use crude enzyme from *Aspergillus niger* and commercial enzymes for the treatment of the pineapple pulp to improve the juice yield with maximum clarity and minimum viscosity and examine the comparative effect of enzymes in crude and purified form by using principal component analysis.

Materials and Methods

Materials Fully ripe fresh pineapple fruits of 'Kew' variety, without any visual blemishes were purchased from local market of Sangrur, Punjab, India. The fruits were washed, peeled, and cut with the help of a knife and were pulped using mixer grinder (MX-113; Maharaja Whiteline, New Delhi, India) to make pulp. The fruit pulp so prepared was used to extract juice.

Crude enzyme preparation *Aspergillus niger* NCIM 548, obtained from the National Chemical Laboratory, Pune, was used for crude enzyme production, since this mold produced a great amount of pectinase, cellulase, and hemicellulase. The organism was maintained on potato dextrose agar slant and sub-cultured every 6-8 weeks. The crude enzyme was produced using this organism under solid state fermentation (SSF) as per the method reported by Kumar *et al*. (7). The crude enzyme so produced was centrifuged at 15,900×*g* for 10 min in centrifuge (RC4100F; Eltek, Elektrocraft(India) Pvt., Ltd., Mumbai, India) to remove all cell mass. The supernatant was lyophilized to concentrate the crude enzyme. The lyophilized enzyme was checked for cellulase and pectinase activity (8) and was filtered through 0.2-μm syringe filter to remove the spores of the fungus to avoid the 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 pineapple pulp to improve the juice yield and quality.

Experimental design and statistical analysis The central composite face centered design (CCD) of the response surface methodology (RSM) was adopted in the experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables and the objective is to optimize this response (9). The main advantage of RSM is reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. The independent variables were the temperature of enzyme treatment (X_1), time of treatment (X_2), and used enzyme concentration (X_3). The variables and their levels were chosen based on the preliminary experiments and literature available on enzymatic hydrolysis of fruits (10) and were as the temperature (X_1; 35-55°C), time (X_2; 210-540 min) of the enzymatic treatment, and concentration of enzyme used (X_3; 0.05-0.15 mL/50 g pulp). The pH of the pulp was adjusted to 4.8 and was excluded from the RSM experimental design. The 3 independent variables were coded as 1 (lowest level), 0, and 1 (highest level). The experimental design matrix in coded (x) form and at the actual level (X) of variables is given in Table 1. The response function (Y) was related to the coded variables by a second degree polynomial equation (Eq. 1) as given below:

$$y=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+ b_{11}x^2{}_1+b_{22}x^2{}_2+b_{33}x^2{}_3+\varepsilon \quad (1)$$

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).

Commercial enzyme treatment of pineapple pulp under optimized conditions The pre-treatment conditions based on the application of commercial enzymes, cellulase from *A. niger* (Fluka BioChemica, Buchs, Switzerland) and pectinase from *A. niger* (Fluka BioChemica) on the juice recovery were also optimized for pineapple ('Kew' variety) in our laboratory using RSM. Temperature, time, concentration of cellulase, and concentration of pectinase in range i.e., 35-55°C, 210-540 min, 6-15 mg/50 g pulp, and

1-2.5 mg/50 g pulp, respectively were adopted as per the existing literature (10) and the preliminary experiments. The %yield, viscosity, and clarity were checked under these conditions.

Analysis of response variables

***Juice yield*:** For each experiment, 50 g of pulp was subjected to different enzyme treatment conditions, as given in Table 1. The temperature was adjusted to the desired level (±0.5°C) by using a high precision water bath (DB-3135S; Decibel, New Delhi, 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 (11) 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*:** The juice was shaken and 10 mL portion of it was centrifuged at 4,800×*g* using centrifuge (RC4100F; Eltek, Elektrocraft(India) Pvt., Ltd.) for 10 min to remove pulp ad coarse cloud particles. The clarity of the juice obtained was determined by measuring the transmittance at a wavelength of 650 nm using UV-VIS spectrophotometer (UV 5704SS; Electronics Corporation of India Ltd., Hyderabad, India) since at this wavelength other browning components do not interfere with the measurements (12). Distilled water was used as a reference. The percent transmittance (%T) 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 Oswald viscometer was noted using a stopwatch for the reference and the sample at 20±2°C (13).

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

where, D=density, t=time of flow, s=sample, w=water.

Optimization and verification Graphical and numerical optimization procedures were used to establish the optimal level of 3 independent variables, temperature (X_1), time (X_2) of the enzymatic treatment, and concentration of enzyme (X_3), resulting to desirable response goals which were maximum yield, maximum clarity, and minimum viscosity. For graphical optimization, a 3 dimensional response surface was plotted by keeping 1 variable constant at the centre point while varying the 2 other variables in the experimental range. For determining the exact optimum value of individual and multiple response, a numerical optimization process was carried out using Design Expert software (Design Expert Software 'DE-6') (trial version; STAT-EASE Inc., Minneapolis, MN, USA). To verify the predicted results, the experiments were performed and the experimental values were compared with predicted one. The validity and adequacy of the predictive models was carried out on the basis of R^2, adjusted R^2, F-value, lack of fit, etc obtained from the analysis of the experimental data by using Design Expert software.

Comparative study using principal component analysis (PCA) The usages of commercial enzymes were compared with the crude enzymes under the optimized conditions, on the same variety of pineapple to look at the feasibility of the application of the crude enzyme in place of commercial enzymes. The MiniTab v16 (MiniTab Inc., Stat College, PA, USA) was used for the PCA to form a smaller number of uncorrelated variables from a large set of data. A large number of variables are reduced to a few variables called principal components (PCs) that describe the greatest variance in the data analyzed. The technique provides an overview of the similarities and differences between the crude and commercial enzymes treated samples and of the interrelationships between the measured properties.

Results and Discussion

Optimization of pre-treatment conditions by using crude enzyme The juice extracted from control and enzyme treated pulp was evaluated for the juice yield (%), viscosity, and clarity. The ranges of different parameters (juice yield, apparent viscosity, and clarity) of enzyme treated samples are shown in Table 1. The data indicated that the quantity and quality of juice has been improved significantly by the enzymatic treatment. The experimental values for all the 3 responses (juice yield, apparent viscosity, and clarity) under different combination of treatment conditions are given in Table 1.

Fitting the model The fitness and adequacy of the model was judged by the coefficient of determination (R^2), which can be defined as the ratio of the explained variation to the total variation. The closer the R^2 value to unity, the better the empirical model fits the actual data. The coefficients of determination, R^2, in the model were 0.9785, 0.9850, and 0.9779 for the regressed models predicting the juice yield, viscosity, and clarity, respectively (Table 2), suggesting a good fit for the models. The predicted models seemed to 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

Table 1. Central composite face centered experimental design employed for enzymatic hydrolysis pretreatment of pineapple

	Coded variables			Uncoded variables			Responses		
	X_1	X_2	X_3	Temp. (°C)	Time (min)	Conc. of crude enzyme (mL)	%Yield	Viscosity (cp)	Clarity (%T)
1	-1	-1	-1	35	210	0.05	71.8	1.77	57.2
2	1	-1	-1	55	210	0.05	70.3	1.78	58.6
3	-1	1	-1	35	540	0.05	79.3	1.59	65.2
4	1	1	-1	55	540	0.05	77.8	1.61	67.4
5	-1	-1	1	35	210	0.15	81.5	1.51	65.2
6	1	-1	1	55	210	0.15	80.9	1.52	66.9
7	-1	1	1	35	540	0.15	83.7	1.45	73.4
8	1	1	1	55	540	0.15	82.9	1.46	74.8
9	-1	0	0	35	375	0.1	82.8	1.47	72.9
10	1	0	0	55	375	0.1	82	1.48	74.5
11	0	-1	0	45	210	0.1	79.3	1.57	66.9
12	0	1	0	45	540	0.1	82.9	1.47	75.3
13	0	0	-1	45	375	0.05	78.8	1.59	66.1
14	0	0	1	45	375	0.15	85.8	1.39	78.5
15	0	0	0	45	375	0.1	86.1	1.43	76.2
16	0	0	0	45	375	0.1	85.1	1.43	77.3
17	0	0	0	45	375	0.1	84.3	1.42	78.4
18	0	0	0	45	375	0.1	85.4	1.4	77.3
19	0	0	0	45	375	0.1	85.6	1.41	76.2
20	0	0	0	45	375	0.1	85.8	1.39	78.4

Table 2. Regression coefficients of predicted quadratic polynomial models for the responses of the model

Coefficients	Juice yield	Viscosity	Clarity
Intercept	84.88*[1)]	1.42*	+76.78*
Linear			
X_1	-0.52***	$+6\times10^{-3}$	+0.83***
X_2	2.28*	-0.06*	+4.13*
X_3	3.68*	-0.1*	+4.43**
Quadratic			
X_1^2	-1.71**	0.04**	-2.30**
X_2^2	-3.01**	0.08*	-4.90**
X_3^2	-1.81**	0.05**	-3.70**
Cross product			
X_1X_2	-0.03	$+1.250\times10^{-3}$	+0.062
X_1X_3	0.2	-1.250×10^{-3}	-0.063
X_2X_3	-1.35**	0.03**	-0.088
R^{2} [2)]	0.9785	0.9850	0.9779
Adj. R^2	0.9591	0.9714	0.9579
CV	1.10	1.28	1.90
F-value	50.51	72.80	49.08

[1)]Statistically significant at *$p<0.001$, **$p<0.05$, and ***$p<0.10$
[2)]R^2, coefficient of multiple determination; Adj R^2, adjusted R^2; CV, coefficient of variance

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) describes 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 1.10, 1.28, and 1.90 of the responses juice yield, viscosity, and clarity, respectively (Table 2), revealed that the experimental results were precise and reliable.

The F-value of 50.51, 72.80, and 49.08 for juice yield, viscosity, and juice clarity, respectively (Table 2), implied that the models were significant ($p<0.001$). The model for the juice yield, viscosity, and clarity of juice can be designed by the coefficients for the predictions of the results.

Response surface analysis

***Juice yield*:** The yield of the juice under different experimental conditions ranged 70.3 to 86.1% as shown in the Table 1. The minimum juice yield was observed under crude enzyme concentration 0.05 mL/50 g, incubation time 210 min, and temperature 55°C. Whereas maximum juice yield was observed at crude enzyme concentration 0.10 mL/50 g, incubation 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. The response surface curves for juice yield are shown in Fig. 1. Each figure demonstrates the effect of 2 factors while the third factor was fixed at 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 crude enzyme (X_3) at middle level i.e., 0.10 mL/50 g of pulp, respectively. The figure indicates that the juice yield increased with the increase in both time and temperature. With further increase in temperature beyond 43.44ºC and incubation time beyond 437.95 min, the juice yield decreased slowly. The increase in juice recovery in enzymatically hydrolyzed pineapple 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 (14,15). The decrease in juice yield with increasing temperature beyond 43.44ºC 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 43.3ºC for 447 min of time.

The interactive effect of concentration of crude enzyme (X_3) and incubation temperature (X_1) to juice yield is shown in Fig. 1B. The data shows that the juice yield increased with increase in temperature and concentration of crude enzyme up to 44.04ºC and 0.15 mL of crude enzyme concentration. The juice yield decreased slowly beyond 44.04ºC, which 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 Kaur *et al.* (10).

***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 obtain the fruit juices. The variation in the viscosity of the juice under enzymatic treatment is given in Table 1 where as the regression coefficients and significance levels of the terms are given in Table 2. The results indicate that viscosity of the juice of pineapple (control sample) was 1.79 cp while it ranged from 1.39 to 1.78 cp in enzymatically treated sample depending on the experimental conditions (Table 1). The viscosity of juice was minimum when the experimental condition, temperature, time, and concentration of crude enzyme were 45ºC, 375 min, and 0.15 mL/50 g of pulp, respectively, whereas it was observed maximum with 55ºC temperature, 210 min of time, and 0.05 mL/50 g of crude enzyme concentration (Table 1). This showed that enzymatic treatment decreased the viscosity. The decrease in juice viscosity in enzymatically hydrolyzed pineapple samples can be attributed to the action of the pectinases. Fruit juices with high viscosity may lead to a few problems during the filtration process (16). The pectinase hydrolyses pectin and cause pectin-protein complexes to flocculate.

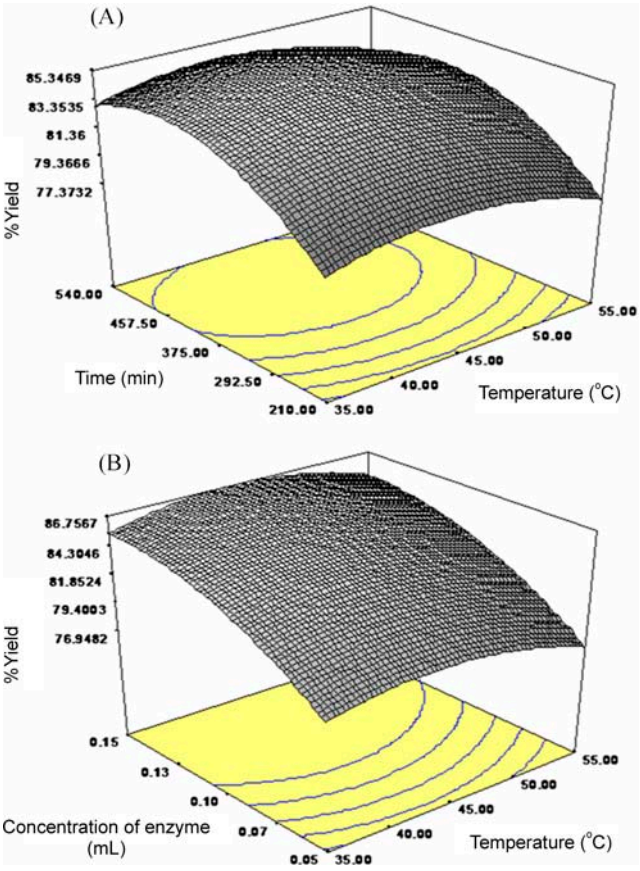

Fig. 1. Response surfaces of juice yield as a function of time and tempersture (A) and concentration of crude enzyme and temperature (B).

The resulting juice from pectinase treatment will have a much lower amount of pectin and a lower viscosity, which facilitates the subsequent filtration processes.

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable (Fig. 2). 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 44.04ºC and 434.39 min, respectively. With further increase in temperature beyond 44.04ºC, the viscosity of juice increased. The findings are in accordance with Rai *et al*. (11), who reported that the viscosity of the mosambi juice decreases with increase in both time and temperature of the enzymatic treatment reaction. The temperature increased the rate of enzymatic reactions.

The interaction effect of incubation temperature (X_1) and crude enzyme concentration (X_3) to viscosity shown in Fig. 2B indicates that the viscosity decreased with increase in concentration of crude enzyme and incubation temperature. The viscosity of juice decreased up to 0.15 mL/50 g of pulp of crude enzyme concentration and temperature

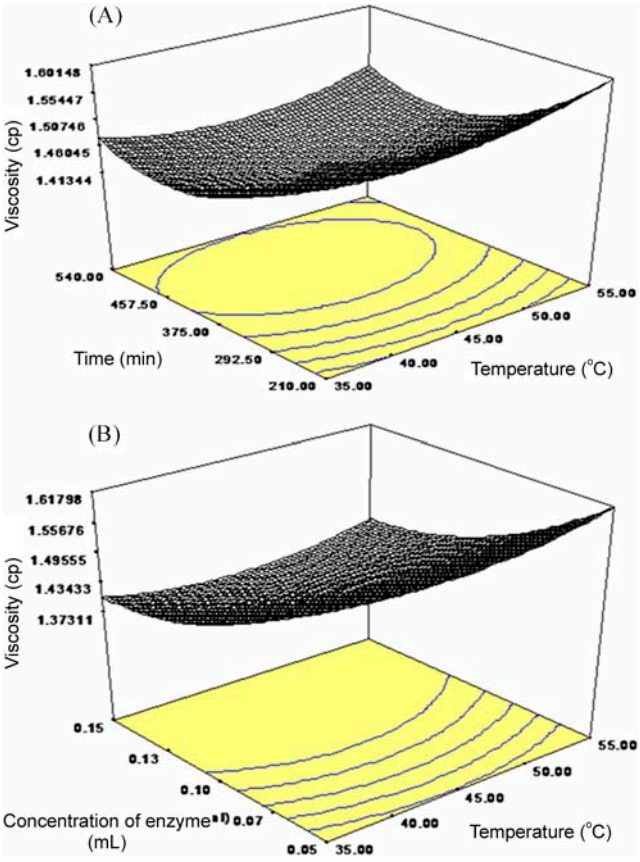

Fig. 2. Response surfaces of viscosity of juice as a function of time and tempersture (A) and concentration of crude enzyme and temperature (B).

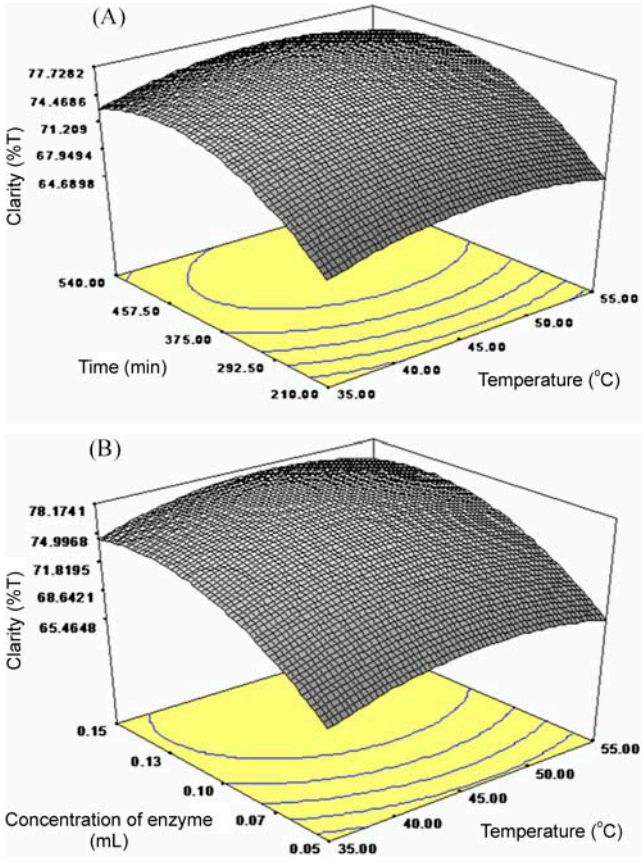

Fig. 3. Response surfaces of clarity of juice as a function of time and tempersture (A) and concentration of crude enzyme and temperature (B).

44.25°C. The juice viscosity increased with further increase in temperature. Lee *et al.* (15) observed that the viscosity of the juice decreases with increase in enzyme concentration up to its maximum value (0.1%).

***Juice clarity*:** The clarity of the juice under different experimental conditions ranged from 57.2 to 78.5%T (Table 1). The minimum clarity was 57.2%T, when the pulp was treated with 0.05 mL/50 g, crude enzyme concentration for 210 min time at 35°C temperature whereas the maximum clarity was observed at crude enzyme concentration 0.15 mg/50 g, time 375 min, and temperature 45°C (Table 1). 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 (17). 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 2).

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable (Fig. 3). It was evident (Fig. 3A) that clarity of juice increased with the increase in both time and temperature up to 445.73 min and 46.80°C, respectively. With further increase in temperature, the clarity of juice decreased. Singh *et al.* (18) observed that the clarity of the Bael juice increased with increase in both temperature and time of the enzymatic reaction. The clarity of juice (Fig. 3B) increased with the increase in both concentration of crude enzyme and incubation temperature up to 0.13 mL/50 g pulp and 46.73°C, respectively. 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 (19). With further increase in the incubation temperature the clarity of juice decreased.

Optimization and verification of process variables The main criterion for constraints was maximum possible juice yield and clarity and minimum viscosity of juice. Under the constraints, the optimum treatment conditions were found to be at incubation temperature 47.28°C, incubation time 445.75 min, and concentration of crude enzyme 0.14 mL/50 g pulp (Table 3). But in practice, it is difficult to

Table 3. Optimization of process variables with respect to juice yield, viscosity, and juice clarity

	Commercial enzymes			Crude enzymes	
	Optimum value (in the range)	Optimum value (targeted)		Optimum value (in the range)	Optimum value (targeted)
Temperature (°C)	46.32	46	Temperature (°C)	47.28	47
Time (min)	447.64	448	Time (min)	445.75	446
Conc. of pectinase (mg/50 g)	2.40	2.40	Conc. of crude enzyme (mL/50 g)	0.14	0.14
Conc. of cellulase (mg/50 g)	13.0	13.0			
	Predicted value	Experimental value		Predicted value	Experimental value
Juice yield (%)	88.41	88.00	Juice yield (%)	86.41	85.90
Viscosity (cp)	1.36	1.36	Viscosity (cp)	1.38	1.38
Juice clarity (%T)	80.97	80.00	Juice clarity (%T)	78.97	78.00

Table 4. Comparison of crude and commercial enzymes for the improvement of juice recovery from pineapple

Parameter	Control	Commercial enzyme treatment		Crude enzyme treatment	
		Treated	Difference	Treated	Difference
Juice yield (%)	70.20	88.0	17.80	85.90	15.70
Viscosity (cp)	1.79	1.36	0.43	1.38	0.41
Juice clarity (%T)	61.1	80.0	18.9	78.0	16.9

maintain the recommended conditions during processing and some deviation is expected. Therefore, optimum conditions were targeted as temperature 47°C, time 446 min, and concentration of crude enzyme 0.14 mL/50 g 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 3). This implied that there was a high fit degree between the observed and predicted values from the regression model.

Optimization of pretreatment conditions using commercial enzymes The optimized conditions for the pre-treatment of pineapple pulp using commercial enzymes were as concentration of pectinase 2.40 mg/50 g of pulp, concentration of cellulase 13.00 mg/50 g of pulp, time 448 min, and temperature 46°C (Table 3). The responses, % juice recovery, viscosity, and clarity were 88, 1.36 cp, and 80%T, respectively, under the optimized conditions (Table 3).

Comparative effect of crude and commercial enzymes for the improvement of juice yield and quality in terms of viscosity and clarity using PCA The effect of crude and commercial enzymes to improve the %yield, viscosity, and clarity of the juice from pineapple were compared under optimized conditions (Table 4). The %yield, viscosity, and clarity of the juice from commercial enzyme treated pulp (under optimized conditions as shown in Table 3) were 88%, 1.36 cp, and 80%T, respectively. The results differed narrowly with the findings of Pal and Khanum (6). The difference may be due to varietal differences or the type and concentration of commercial enzymes used in the study.

The juice yield, viscosity, and clarity from the crude enzyme treated pulp were 85.90%, 1.38 cp, and 78.00%T, respectively, under the optimized conditions. The data (Table 4) indicates that the crude enzyme treatment is equally competitive to the commercial enzymes. The competitiveness of the crude enzyme may be a cumulative effect of other polysaccharases such as hemicellulases along with the pectinases and cellulases present in the crude enzyme. In addition, the usage of crude enzyme will drastically reduce the cost of the enzyme to the proportion of 60-70%. However, the detailed study of the enzymatically treated pineapple juices with respect to its sensory, physicochemical, and microbiological study is required to be undertaken.

PCA was used to form a smaller number of uncorrelated variables from a large set of data. The goal of principal components analysis is to explain the maximum amount of variance with the fewest number of principal components. The results of the analysis are shown in Fig. 4. With this statistical method, a large number of variables are reduced to a few variables called principal components (PCs) that describe the greatest variance in the data analyzed. The PCA plots provide an overview of the similarities and

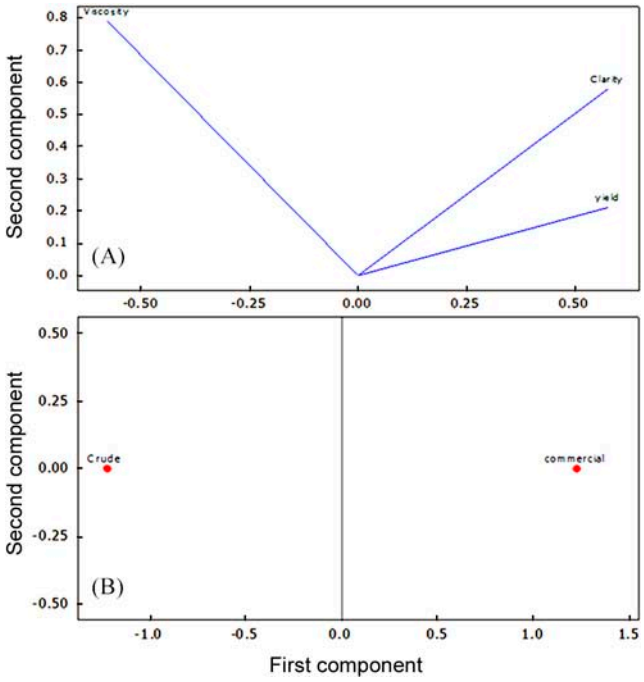

Fig. 4. Loading plot (A) and score plot (B) of yield, clarity, and viscosity of the juice under crude and commercial enzyme treatment.

differences between the crude and commercial enzyme treated samples and of the interrelationships between the measured properties. The distance between the locations of both the samples on the score plot is directly proportional to the degree of difference or similarity between them (Fig. 4B).

From score plot (Fig. 4B) it is clear that commercial enzyme treated sample shows maximum coefficient of variance in the 1st principle component where as crude enzyme treated sample shows maximum coefficient of variance in the 2nd principle component. Hence the variables of commercial enzyme treated samples can be called as the 1st principle components. Maximum covariance of 58% in the 1st principle component was found between clarity and yield where as in the 2nd principle component a variance of 21, 79, and 58% were found for yield, viscosity, and clarity, respectively. In the correlation matrix the maximum Eigen value was 3, which was nearest to the variance derived from viscosity hence the variable viscosity in the 2nd principle component can be denoted as significant variable to distinguish between crude and commercial enzymes treated juices. The loading plot of variables reflects that clarity and yield are correlated to higher degree in comparison to the correlation of these 2 variables with viscosity. This analysis suggest the use of only 2 variable i.e., clarity and yield to be studied for comparing samples of juice treated with same kind of enzyme.

In conclusion, pineapple 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. The comparison of crude and commercial enzymes for the improvement of juice yield, viscosity, and clarity showed that the crude enzyme is competitive to the commercial enzyme for the pineapple pulp treatment to improve juice yield and clarity. The principal component analysis suggests that the juice yield and clarity are the responses to be studied while comparing the different samples treated with commercial enzymes, whereas juice viscosity is the parameter to be considered for comparison of crude and commercial enzyme treated samples. The study indicates that the crude enzyme treatment is equally competitive to the commercial enzymes and thus the use of crude enzyme may be one of the remedies to reduce the processing cost.

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