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Optimization of an enzyme assisted banana pulp clarification process

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Abstract

The study was initiated to optimize the enzymatic clarification process of banana pulp of maturity stage 6 using response surface methodology. Banana pulp was treated with pectinase enzyme (Pectinex Ultra SP-L) at various concentrations (0.05-0.15%), incubation temperature (30-50°C) and time (60-180 min) of treatment. The effect of these enzymatic conditions on per cent juice yield, viscosity of banana pulp and clarity of banana juice were studied by employing Central Composite Design. 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 = 0.9851, 0.9883 and 0.9679, respectively. Based on response surface and desirability graph, the optimum conditions for clarification of banana pulp were: 0.12% enzyme concentration, 38.84°C incubation temperature and 136.52 min of incubation time resulted juice yield, viscosity of pulp and clarity of juice under above conditions were 64.76%, 450.20 cps and 84.56%, respectively.

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Introduction

Generally, fruit juices are extracted by simple crushing and or grinding of fruits. However, in case of banana this process results in a sticky, lumpy mass with no juice. Bananas are usually too pulpy and pectinaceous to yield juice by simple pressing or centrifugation (Adao and Gloria, 2005). Of the problems associated with banana pulp processing, a high viscosity seems to be the most severe. The turbidity and viscosity of banana juice are caused mainly by the polysaccharides in the juice such as pectin and starch (Lee *et al.*, 2006). Pectin makes the clarification process harder because of its fibre-like molecular structure. Starch is also a common problem for juice processors. Polymeric carbohydrates like starch may make filtration difficult and cause post-process cloudiness.

Application of enzymes such as pectinase and amylase improved the clarification process for banana fruit juices (Koffi *et al*., 1991; Yusof and Ibrahim, 1994; Lee *et al*., 2006). Pectinase hydrolyzes pectin and causes pectin–protein complexes to flocculate. The resulting juice has a much lower amount of pectin and a lower viscosity, so any subsequent clarification process is shortened. The enzymatic clarification is influenced by a number of variables including concentration of the enzyme, temperature and incubation time of the treatment (Baumann, 1981; Lanzarini and Pifferi, 1989; Ranveer *et al*., 2013).

Despite various reports on enzymatic depectinization, no work on the optimization of pectinase and cellulase treatment with respect to ripening or maturity stages of banana fruit is reported. Most of the researchers used fully ripened stage i.e. stage 7 for enzymatic clarification study. During ripening as there is drastic change in the composition of pulp viz. starch, sugar content (Kajuna *et al*., 1997; Prabha and Bhagyalakshmi, 1998); enzyme requirement for clarification will also be vary at different ripening stages. Hence, in the present investigation bananas of stage 6 (Tapre, 2013) has been selected to optimize the enzymatic conditions viz. enzyme concentration, temperature and incubation time using response surface methodology (RSM).

Materials and Methods

Bananas (*Musa* sp.) of variety 'Robusta' were procured at the maturity stage 1 (all green) from selected banana orchards near Anand city (India). The procured material was sorted, tagged and kept for ripening as per the method suggested by Kulkarni *et al.* (2010) up to 6th stage of maturity (all yellow) according to Standard Banana Colour Chart. Commercial pectinolytic enzyme i.e. Pectinex Ultra SP-L (with enzymatic activity 26000 PG U per ml, optimum pH 3.5 to 6 and temperature below 50°C) and cellulase enzyme i.e. Celluclast 1.5L with cellulose activity 158 U per ml were obtained from Novozymes South Asia Pvt. Ltd., Bangalore.

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Table 1. The central composite experimental design employed for enzymatic clarification of banana juice

Sr. No.	Enzyme Concentration (%)	Temperature (^{0}C)	Time (min)
	X_1 (x_1)	X_2 (x_2)	X_3 (x_3)
1	0.15(+1)	50(+1)	60(-1)
2	0.1(0)	40(0)	120(0)
3	0.15(+1)	30(-1)	180(+1)
4	0.1(0)	40(0)	120(0)
5	0.05(-1)	50(+1)	180(+1)
6	0.05(-1)	30(-1)	60(-1)
7	0.05(-1)	30(-1)	180(+1)
8	0.1 (0)	40(0)	120(0)
9	0.1(0)	40(0)	120(0)
10	0.05(-1)	50(+1)	60(-1)
11	0.15(+1)	50(+1)	180(+1)
12	0.15(+1)	30(-1)	60(-1)
13	0.1(0)	40(0)	120(0)
14	0.15(+1)	40(0)	120(0)
15	0.1(0)	40(0)	120(0)
16	0.1(0)	50(+1)	120(0)
17	0.1(0)	40(0)	60(-1)
18	0.05(-1)	40(0)	120(0)
19	0.1(0)	40(0)	180(+1)
20	0.1(0)	30(-1)	120(0)

x represents the coded level of variables.
X represents the actual level of variables.

Banana pulp clarification process

Banana pulp of maturity stage 6 has been clarified by using the commercial pectinolytic enzyme i.e. Pectinex Ultra SP-L and cellulase enzyme i.e. Celluclast 1.5L. The banana fruits were washed, peeled manually and cut into small pieces. Pieces were then pulped using a kitchen blender for 2 minute into pulp. After the pulping, the pulp was divided into equal portions for enzymatic treatment. For each experiment 500 g pulp was subjected to different pectinase enzyme treatment conditions as shown in Table 1.

The required quantity (0.05-0.15%) of pectinase enzyme i.e. Pectinex Ultra SP-L pulp was added to 500 g batches of banana pulp and incubated at different temperature (30-50^{o}C) and time (60-180 min) and then treated with 0.05% cellulase enzyme i.e. Celluclast 1.5L. At the end of the treatment, the enzymes in the pulp was inactivated by heating at 90^{o}C for 5 minute and immediately cooled to room temperature. At this stage viscosity of the pulp samples were measured. The enzyme treated pulp was centrifuged at 2900 g/15 minute. The supernatant was filtered through a fine mesh nylon cloth spread on glass funnel and juice was collected. Viscosity of treated pulp, yield and clarity of juice were used as basis for optimization study.

Juice yield was estimated as percentage of the juice obtained based on the initial pulp. Viscosity of clarified banana juice was determined by using a Brookfield Viscometer (Model LVDV-II+, Brookfield Engineering Laboratory, Inc.) at 100 rpm and 30^{o}C temperature with spindle No. LV3 and LV4. Clarity of the juice obtained was determined by measuring % transmittance at a wavelength of 660 nm using 'Shimadzu UV–VIS' spectrophotometer. Distilled water was used as the reference.

Experimental design and statistical analysis

Response Surface Methodology (RSM) was used to generate the experimental designs, statistical analysis and regression model with the help of Design Expert Software Version 8 (Statease Inc.). The Central Composite Design (CCD) with a quadratic model (Box and Draper 1987) was employed. Three independent variables namely enzyme concentration (x1), temperature (x2) and time (x3) were chosen. Each independent variable had 3 levels which were coded as (-) 1, 0 and (+)1. A total of 20 different combinations (including six replicates of the of the centre point each signed the coded value 0) were chosen in random order according to a CCD for three factors (Cochran and Cox, 1957). The experimental design in the coded (x) and actual (X) levels of variables is shown in Table 1. The responses function (y) measured were the yield, viscosity and clarity of the banana juice. These values were related to the coded variables (xi, i = 1, 2 and 3) by a second degree polynomial using the equation below.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value and compared with standard significance level of 0.1%, 1% and 5%. The regression coefficients were then used to make statistical calculation to generate contour maps from the regression models.

Results and Discussion

Enzymatic clarification of banana pulp of 6th stage of maturity has been studied by applying different enzymatic treatment conditions viz. enzyme concentration, temperature and time. The experimental values for responses viz. yield, viscosity and clarity under different treatment conditions are presented in Table 2. The regression coefficients for second order polynomial equations and results for linear, quadratic and interaction terms are presented in Table

Table 2. Effect of enzyme concentration, temperature and time on three dependent variables for pulp of maturity stage 6

Run No.	*Factors*			*Responses*		
	Enzyme Concentration (%)	Temp (^{0}C)	Time (min)	Yield (%)	Viscosity (cps)	Clarity (% T)
1	0.15	50	60	50.78	670.68	61.46
2	0.1	40	120	62	502.78	82.14
3	0.15	30	180	58.08	570.42	72.58
4	0.1	40	120	62.42	505.6	82.18
5	0.05	50	180	44.04	830.45	50.44
6	0.05	30	60	38.8	995.16	50.19
7	0.05	30	180	42.02	925.12	59.8
8	0.1	40	120	64.56	480.46	85.28
9	0.1	40	120	63.04	506.2	83.16
10	0.05	50	60	38.7	890.64	47.45
11	0.15	50	180	56.1	600.28	64.12
12	0.15	30	60	50.8	670.22	58.12
13	0.1	40	120	59.78	510.8	81.24
14	0.15	40	120	64.02	480.48	82.54
15	0.1	40	120	59.22	510.42	81.18
16	0.1	50	120	55.32	610.12	68.43
17	0.1	40	60	52.12	650.12	62.1
18	0.05	40	120	48.9	702.14	60.1
19	0.1	40	180	61.02	490.9	77.12
20	0.1	30	120	56.02	632.64	70.42

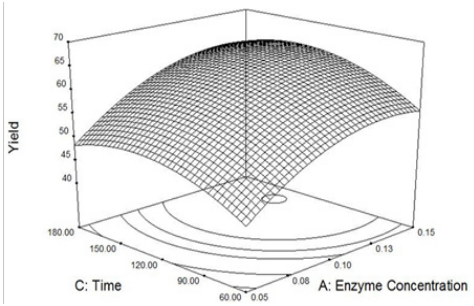

Figure 1. Response surface for yield of banana juice as a function of enzyme concentration and time (at temp 40^{o}C)

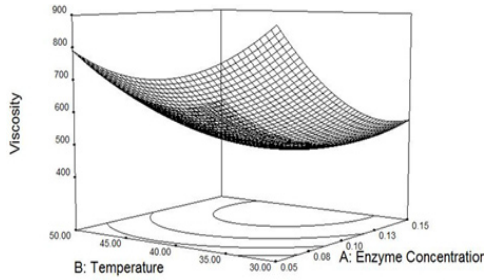

Figure 2. Response surface for viscosity of banana pulp as a function of enzyme concentration and temperature (at time 120 min)

3. The statistical ananlysis indicates that the proposed model was adquate, possessing no lack of fit and with satisfactory values of the R^2 for all the responses. The R^2 values for yield, viscosity and clarity were 0.9851, 0.9883 and 0.9679, respectively.

To aid visualization, the response surfaces for yield, viscosity and clarity are shown in Figs. 1 to 3.

Effect of enzyme concentration, temperature and incubation time on yield of banana juice

Regression model (Table 3) showed that the yield was significantly affected by linear effects of enzyme concentration and incubation time ($p < 0.01$). The linear effect of both these parameters viz. enzyme concentration and time were positive whereas the quadratic effect were found to be significantly negative ($p < 0.01$), thus resulting in a curvilinear increase in yield for all incubation times. In addition, the quadratic effect of temperature also significantly affected on yield of juice ($p < 0.01$). These results support the finding of Rastogi and Rashmi (1999) who reported juice yield as a function of linear and quadratic effects of enzyme concentration and time.

The interaction effect of enzyme concentration and time can be seen from Figure 1. Upto a certain level of increased in enzyme concentration, the yield of juice also gets increased and then further no increased in yield was observed. Similar effect of incubation

Table 3. Regression coefficients, R^2 and mean and SD value for dependant variables

Regression coefficient	Yield (%)	Viscosity (cps)	Clarity (%)
b_0	62.14	501.72	82.11
b_1	6.73*	-135.14*	7.08*
b_2	-0.078	-19.14**	-1.92
b_3	3.01*	-45.97*	4.47*
b_{12}	-0.49	28.69**	0.87
b_{13}	0.51	-5.00	0.57
b_{23}	0.02	4.91	-2.30***
b_{11}	-4.86*	86.66*	-7.35*
b_{22}	-5.65*	116.73*	-9.24*
b_{33}	-4.75*	65.86*	-9.06*
R^2	0.9851	0.9883	0.9679
Mean	54.39	636.78	69.00
SD	1.51	25.05	3.32

Subscripts 1= Enzyme concentration, 2= Temperature, 3= Incubation time
* Significant at 0.01 level
** Significant at 0.05 level
*** Significant at 0.1 level

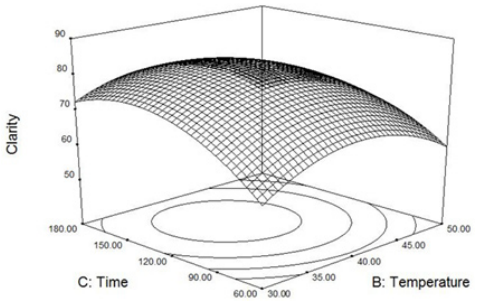

Figure 3. Response surface for clarity of banana juice as a function of temperature and time (at enzyme concentration 0.1%)

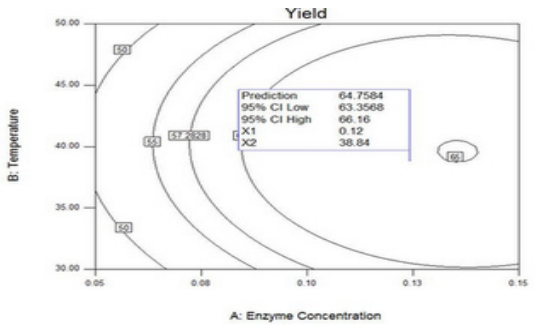

Figure 4. The contour plots of yield of banana juice as a function of enzyme concentration, temperature and time (at time 136.52 min)

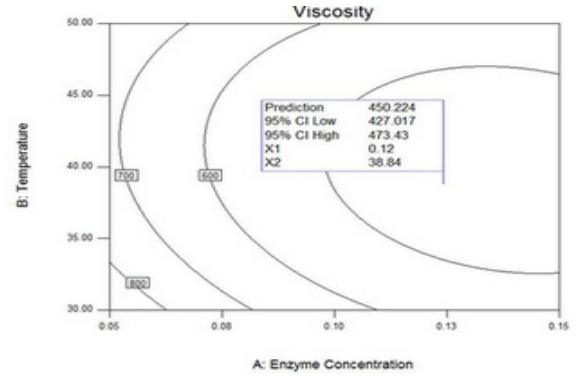

Figure 5. The contour plots of viscosity of banana juice as a function of enzyme concentration, temperature and time (at time 136.52 minute)

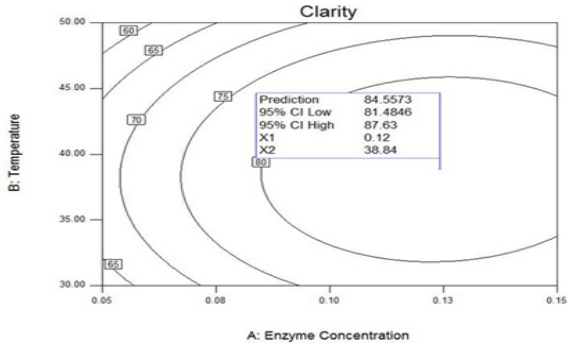

Figure 6. The contour plots of clarity of banana juice as a function of enzyme concentration, temperature and time (at time 136.52 min)

time was observed on yield of banana juice. This increased in juice yield due to enzymatic treatment is mainly influenced by the de-polymerization and de-esterification effect of pectinase enzyme on the complex pectin molecules, degrading cell walls of pectinaceous nature and thus enabling easy release of fruit juice. The results of this study concur with those other results previously reported (Kilara 1982; Koffi *et al*., 1991; Yusof and Ibrahim 1994; Tadakittisarn *et al.*, 2007) and confirm the significant role played by pectinolytic enzyme in influencing banana juice yield.

Effect of enzyme concentration, temperature and incubation time on viscosity of banana pulp

It can easily be accessed from the Table 3 that the viscosity was negatively related to the linear effects of enzyme concentration ($p < 0.01$), temperature ($p < 0.05$) and incubation time ($p < 0.01$) while positive relationship with quadratic effects of enzyme concentration, temperature and incubation time ($p < 0.01$) was observed. The interaction effect of enzyme concentration and temperature was also significant ($p < 0.05$).

As enzyme concentration had a negative effect on viscosity at linear terms, viscosity was significantly reduced with higher enzyme concentration as

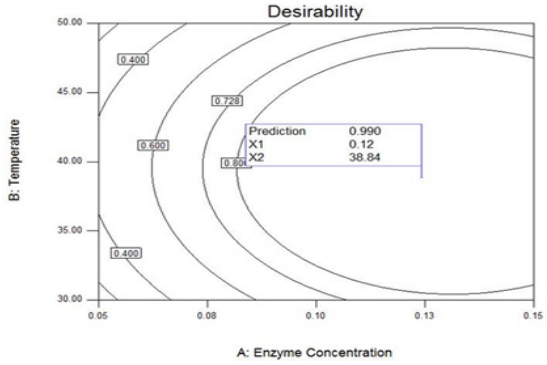

Figure 7. Desirability graph as a function of enzyme concentration, temperature and time (at time 136.52 min)

observed in Figure 2. Whereas increased in temperature (up to 40°C) the viscosity of the juice decreased but further increased in temperature increased the viscosity. This may be due to denaturation of enzymes at high temperature.

The increase in temperature during the enzyme treatment contributed to pectin cells destruction, and the enzyme activity was reduced when the incubation temperature was high, therefore, the enzyme action on pectin cell was also reduced and this contributed to the increase in viscosity (Karangwa *et al.*, 2010). The decreased in the viscosity of fruit juices due to enzymatic hydrolysis of pectin has been reported by Urlaub (1996).

Effect of enzyme concentration, temperature and incubation time on clarity of banana juice

From Table 3, it is revealed that clarity depends on enzyme concentration and incubation time as their linear effect was positive at $p < 0.01$. 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 (Shahaden and Abdullah, 1995; Sin *et al.,* 2006). The quadratic effect of all the variables (enzyme concentration, temperature, incubation time) was negative at $p < 0.01$. It can also be seen in Table 3 that there is only a significant interaction effect between temperature and incubation time at $p < 0.1$ with a negative effect, therefore the incubation time depends on enzyme concentration.

Figure 3 shows the 3-Diamentinal Graph for the effect of independent variables on clarity. It can be concluded from Figure 3 that the clarity of juice increased with increase in incubation time up to certain level then it stabilized. Increased in temperature (up to 40°C) increased the clarity but further increased in temperature gradually decreased the clarity. The temperature increases the rate of

enzymatic reactions, hence the rate of clarification, as long as the higher temperature adversely affects the activity of enzyme.

Optimization of process parameters for clarified banana juice

The applied enzymatic conditions viz. enzyme concentration, temperature and incubation time for the clarification of banana pulp of stage 6 were optimized by Numerical Optimization. The criterions applied for numerical optimization were maximum yield, minimum viscosity and maximum clarity. The criteria set above produced the graphs of optimum enzymatic conditions of the clarification process for respective responses viz. yield, viscosity and clarity as shown in Figures (4 to 6) and the overall desirability graphs of the most desirable combination for clarification of banana pulp at stage 6 as shown in Figure 7.

Figure 4 indicates that increased in enzyme concentration increased the yield of banana juice. At optimum conditions of enzyme concentration (0.12%), temperature (38.84^{o}C) and time (136.52 min) the maximum yield 64.75 ± 1.40 per cent could be obtained. At these optimum conditions the yield and clarity obtained were maximum whereas minimum viscosity of the treated pulp was obtained.

Figure 5 depicts the contour plots of viscosity of enzymatic treated banana pulp as a function of process variables. Viscosity of treated banana pulp could be decreased to minimum level of 450.22 ± 23.20 cps with optimum conditions of enzyme concentration (0.12%), temperature (38.84^{o}C) and time (136.52 min).Maximum clarity of 84.56 ± 3.07% can be obtained in clarified banana juice at same optimum enzymatic conditions those recorded for the above two response i.e. yield and clarity (Figure 6).

Figure 7 shows the desirability graph for the most desirable enzymatic combination for clarification of banana pulp at stage 6. The process variables for the best combination of response functions were 0.12% enzyme concentration, temperature 38.84^{o}C and incubation time 136.52 min. The response functions were calculated from the final polynomial and the response at this optimized combination were yield (64.76%), viscosity (450.20 cps) and clarity (84.56%). For experimental purpose, the above optimum enzymatic conditions were considered as: 0.12% enzyme concentration, temperature 40.0^{o}C and incubation time 135 minutes. Slight variations in these optimized enzymatic combinations were observed as compared to those recommended by previous researchers (Kotecha *et al.*, 1994; Shahadan and Abdullah, 1995). This may be due to utilization of bananas of different ripening stages and type of enzymes utilized during clarification study.

Conclusion

The present study concluded that viscosity of banana pulp, yield and clarity of banana juice are the functions of different enzymatic treatment conditions viz. enzyme concentration, incubation temperature and time. Statistical analysis using RSM appeared to be a valuable tool for optimizing the effects of applied enzymatic treatment conditions on enzymatic clarification of banana pulp. The recommended enzymatic clarification condition for the banana pulp of maturity stage 6 was 0.12% enzyme concentration at 38.84^{o}C for 136.52 min to achieve maximum juice yield (64.76%) and clarity (84.56%) and minimum pulp viscosity (450.20 cps).

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