

Response Surface Optimization Of Banana (Musa Sp.) Fruit Juice Clarification Using Multi-Enzyme System (Pectinase, Cellulase And Hemicellulase)

Ravi Kumar^{1*}, Alak Kumar Singh², Sunil Kumar Yadav³,
Mahendra Kumar⁴

^{1*}Department of Food Technology, Institute of Engg. & Technology,
Bundelkhand University, Jhansi 284128, India. e-mail:
ravihbti15@yahoo.com

²Department of Food Technology, School of Chemical Technology, Harcourt
Butler Technical University, Kanpur-208002, India. e-mail:
alakksingh@rediffmail.com

³Department of Chemistry, SRM Institute of Science and Technology, Delhi-
NCR, Campus Ghaziabad-201204, India. e-mail: skyhbtu@gmail.com

⁴National Center for Flexible Electronics, Indian Institute of Technology
Kanpur, Kanpur- 208016, India.e-mail: mahendra.bbt@gmail.com

Abstract

This study was carried out with an objective of producing high quality clarified banana juice with high yield by multienzyme treatment. Banana juice was treated with various concentration levels of commercial enzymes pectinase, cellulase and hemicellulase for different incubation periods. The effect of enzyme treatment conditions was studied on turbidity and yield of juice obtained from banana juice and optimum process conditions were determined. Response Surface Methodology (RSM) employing a second order central composite design was used to obtain optimum process conditions for simultaneous treatment with the range of variables for enzymatic treatment conditions giving the optimum values as 0.07%, 0.46% and 0.79% enzyme concentration for Pectinase, Cellulase and Hemicellulase respectively and incubation period of 123 min, at incubation temperature of 55 °C. Under this condition, the juice was obtained with turbidity value as 7 NTU and 79.5% of yield.

Introduction

Banana is a tropical fruit of high nutritional quality

with its widely appreciated flavour and aroma. It is one of the abundant and cheap fruits in India. The country ranks first in production of Bananas (22.94%) (APEDA, 2019). However, only 0.05% of production of banana is exported and the rest is consumed within the country as table fruit. Although, in international trade banana is the most popular one and ranks second after citrus fruits in terms of value, India largest banana producing country is hardly involved in it. The causes behind low volume export of banana include non-ideal post-harvest practices, transport practices, improper storage facilities, and outdated banana handling practices. Because of mishandling of produce about 25-40% is being wasted and only 2% is processed into value added products (NHB, 2017). So there is ample scope for production of high-value clarified juices from banana to minimize the wastage and to earn higher foreign revenue by increasing the export of such valuable products.

Fruit juices are generally extracted by crushing and grinding. Juices obtained by these operations are viscous, turbid and cloudy. This happens due to the presence of pulp particles and colloidal suspensions. Yield of this kind of juices is low and it is very difficult to concentrate and pasteurize them. In case of banana also crushing and grinding do not yield juice from banana as bananas are too pulpy and pectinaceous (Adao and Gloria, 2005). A sticky and lumpy mass is obtained after these operations with banana. The most severe problem associated with banana pulp processing is high viscosity. The viscosity and turbidity of banana juice are caused mainly by the polysaccharides in the juice such as pectin and starch (Lee et al., 2006). Juice clarification is very important process for juice processing industry because it enhances the acceptability of the product (Sharma et al., 2015). Juices with unacceptable cloud and muddy turbidity are undesirable for marketing. (Shah & Nath, 2007). For the preparation of ready to serve drinks, jelly, cordials, nutritional carbonated beverages, concentrates, nectars, etc., clarified juices are in high demand (Brito et al., 2008). A variety of products based on clarified juice such as sparkling clear beverages (soft drinks, clear juice cocktails, alcoholic beverages, cold teas with clear juice),

translucent jelly products, candies, clear juice blends, fruit honey or fruit sugar, 100% canned fruit (with clear juice as syrup) etc. are making place in the market. This shows that there exist several market opportunities, not only for the traditional clear juice from apple, but also for clarified juices produced from fruits with high pulp content (Vaillant et al., 2001). As a crop, a large percentage of banana is not suitable for the fresh market since it is too mature for shipment (Koffe et al., 1991; Sims et al., 1994). Bananas have a high sugar content and a recognizable, desirable flavour. High-value clarified juices from these excess bananas could become valuable products from otherwise rejected bananas (Sims and Bates, 1994). Clarification is the process of breaking the semistable emulsion of colloidal plant carbohydrates that support the insoluble cloud material in a freshly extracted juice. Enzymatic treatment of juices results in degradation of pectin and viscosity reduction which facilitates separation through filtration or centrifugation giving the juice higher clarity. Currently pectinases, cellulases and hemicellulases, collectively called macerating enzymes are used for extraction and clarification of fruit juices Sharma et al., 2016, Tapre & Jain, 2014, Sagu et al., 2014, Cheirsilp & Umsakul, 2008, Tadakittisarn et al., 2007 and Lee et al., 2006, studied the effect of pectinase enzyme for clarification of banana pulp.

The fruit-based juice market is one of the fastest-growing categories in the beverage segment. It is growing at a CAGR (compound annual growth rate) of 25-30 per cent in the past decade. In India, the juice market is estimated to be around Rs 10,781.62 crore (16 per cent of the total soft drink market in India). The juice business in India is highly dominated by unorganised players having a market share of over 75 per cent. The organised retail has only 25 per cent of the business and comprises juice bars, juice cafes and packaged juice players. The fruit juice market in India is projected to grow at a compound annual growth rate of 22 per cent over the next five years, and is expected to grow more than double in the next few years (Food and Beverage News, 2016).

Materials and Methods

Fruits: Fresh and mature bananas (*Musa* sp.) were purchased from the local market and used immediately or stored at 4 °C for not more than 2 days before being used.

Enzyme Source: Commercial enzymes, BL-Pectinase, BL-Cellulase and BL-Hemicellulase obtained from Biolaxi Corporation, Bhiwandi, India, were used for enzymatic treatment of fruit juice. BL-Pectinase is a food grade enzyme preparation specially designed for cell wall degradation and pectic substance extraction. The activity of BL-Pectinase is 1200 PGU/g. The recommended optimum enzyme reaction conditions are at pH 3.5 to 6.0 (Optimum 3.8) and temperature 40 °C to 60 °C (Optimum 55 °C). BL-Cellulase and BL-Hemicellulase are also food grade enzyme preparations designed for cell wall degradation and extraction with the activities of 1,00,000 CMCU/g and 1,00,000 HCU/g respectively. The recommended optimum enzyme reaction conditions for BL-Cellulase are at pH 4.5 to 6.0 (Optimum 4.8) and temperature 40 °C to 60 °C (Optimum 55 °C) and for BL-Hemicellulase are at pH 4.5 to 6.5 (Optimum 4.5) and temperature 40 °C to 60 °C (Optimum 50 °C).

Juice Preparation: Mature bananas were washed, peeled and cut into small pieces. Based on previous works, a ratio of 1:2 (Banana: water; w/w) (Sagu et al., 2014) was used in pulping using an electric blender. The juice obtained was subjected to different enzyme treatment conditions. The pH of the juice obtained was 5.1. Figure 1 shows the steps involved in extraction and clarification of banana juice by enzyme treatment.

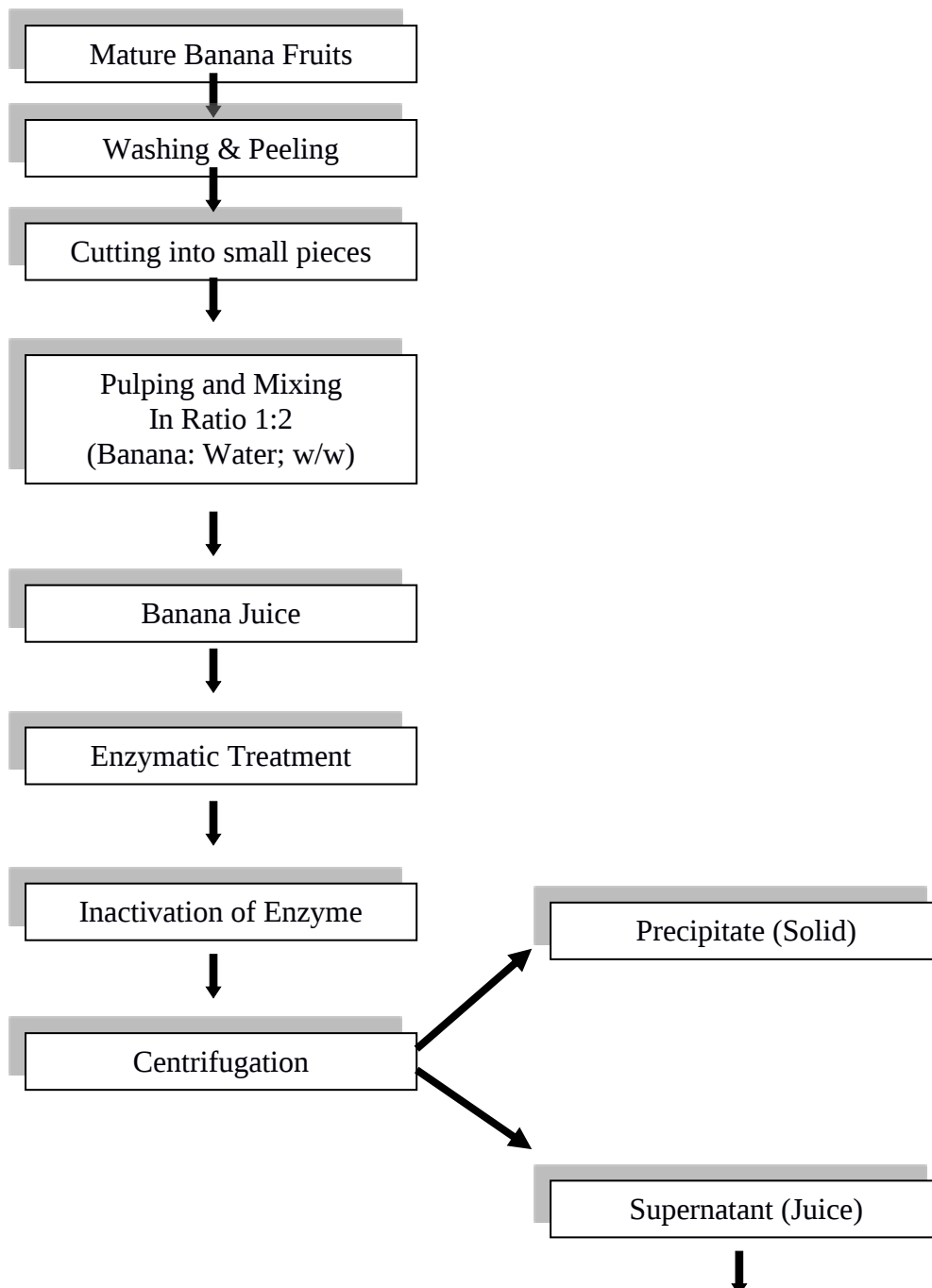
Enzymatic Treatment and Optimization:

To obtain optimum process conditions for simultaneous treatment of all the three enzymes by Response Surface Methodology (RSM), a four variable (five level of each) second order central composite rotatable design (CCRD) was employed. The independent variables were concentration of pectinase (x1), concentration of cellulase (x2), concentration of hemicellulase (x3) and incubation

time (x4). Based on the above mentioned experiments with the individual enzymes, the ranges of variables are selected as follows:

1. Enzyme Concentration (Pectinase), X1: 0.00-0.12% w/w
2. Enzyme Concentration (Cellulase), X2: 0.00-0.80% w/w
3. Enzyme Concentration (hemicellulase), X3: 0.00-1.40% w/w
4. Incubation Time, X4: 60-180 min

The experimental design is shown in Table-1 in coded (x) and actual (X) levels of variables.



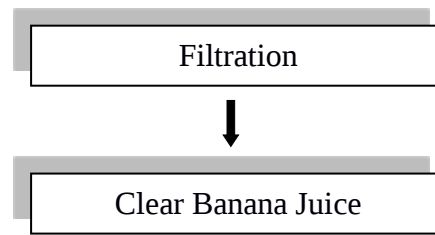


Fig 1: Steps for Banana Juice Extraction and Subsequent Clarification by Treatment with Enzymes

Table-1: The Central Composite Rotatable Experimental Design (in coded and actual level of four variables) employed for treatment of Banana juice with enzymes

Central Composite Design

Factors: 4 Replicates: 1
 Base runs: 31 Total runs: 31
 Base blocks: 1 Total blocks: 1

Two-level factorial: Full factorial

Cube points: 16

Center points in cube: 7

Axial points: 8

Center points in axial: 0

α : 2

Design Table

Experiment No.	Enzyme Concentration (% w/w)						Incubation Time (min)	
	Pectinase		Cellulase		Hemicellulase			
	X ₁	x ₁	X ₂	x ₂	X ₃	x ₃	X ₄	x ₄
1	0.09	1	0.6	1	0.35	-1	150	1
2	0.06	0	0.4	0	0.70	0	60	-2
3	0.09	1	0.6	1	0.35	-1	90	-1
4	0.06	0	0.4	0	1.40	2	120	0
5	0.06	0	0.4	0	0.70	0	120	0
6	0.03	-1	0.2	-1	1.05	1	90	-1
7	0.12	2	0.4	0	0.70	0	120	0
8	0.00	-2	0.4	0	0.70	0	120	0
9	0.06	0	0.4	0	0.70	0	120	0
10	0.03	-1	0.6	1	1.05	1	90	-1
11	0.09	1	0.6	1	1.05	1	90	-1
12	0.09	1	0.2	-1	1.05	1	150	1
13	0.03	-1	0.6	1	0.35	-1	90	-1
14	0.03	-1	0.2	-1	0.35	-1	90	-1
15	0.06	0	0.4	0	0.70	0	120	0
16	0.09	1	0.2	-1	1.05	1	90	-1

17	0.06	0	0.0	-2	0.70	0	120	0
18	0.09	1	0.6	1	1.05	1	150	1
19	0.06	0	0.4	0	0.70	0	120	0
20	0.03	-1	0.2	-1	0.35	-1	150	1
21	0.09	1	0.2	-1	0.35	-1	150	1
22	0.06	0	0.4	0	0.70	0	180	2
23	0.06	0	0.4	0	0.00	-2	120	0
24	0.03	-1	0.6	1	0.35	-1	150	1
25	0.03	-1	0.6	1	1.05	1	150	1
26	0.06	0	0.8	2	0.70	0	120	0
27	0.06	0	0.4	0	0.70	0	120	0
28	0.03	-1	0.2	-1	1.05	1	150	1
29	0.06	0	0.4	0	0.70	0	120	0
30	0.09	1	0.2	-1	0.35	-1	90	-1
31	0.06	0	0.4	0	0.70	0	120	0

The response functions (y) were the turbidity and juice yield. The values were related to the coded variables (x_i , $i=1, 2, 3$) by a second-degree polynomial using the equation below (Eq. 1):

$$Y_k = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{33}X_3X_3 + b_{44}X_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + \epsilon$$

[1]

The coefficients of polynomial were represented by constant term b_0 ; linear effects b_1 , b_2 , b_3 and b_4 ; quadratic effects b_{11} , b_{22} , b_{33} and b_{44} ; interaction effects b_{12} , b_{13} , b_{14} , b_{23} , b_{24} and b_{34} and random error ϵ . The Analysis of Variance tables were generated and the effect of individual linear, quadratic and interaction terms were determined. The significance of all the terms in the polynomial was judged statistically by computing the probability (p) at level of 0.001, 0.01 and 0.05. Minitab 17.1.0 (Minitab Inc.) statistical software was used for data analysis.

Turbidity Determination: Turbidity was determined using Insif India Digital Turbidity Meter (Labpro International, Ambala, Haryana). The results were reported as Nephelometric Turbidity Units (NTU).

Percentage Yield Determination: Percent yield was estimated as percentage of the clarified juice obtained based on the initial fruit pulp.

Results & Discussion

Response Surface Optimization

The experimental results on the effect of the independent variables viz. concentrations of pectinase, cellulase and hemicellulase and incubation time on the responses (turbidity and juice yield) are shown in Table 2. The regression coefficients and R^2 values for second order polynomial equation are presented in Table 3 for the responses. The adequacy and fitness of these equations were tested by analysis of variance (ANOVA) (Khuri and Cornell, 1989). Table 4 shows Analysis of Variance of regression models for responses. It suggests that linear and quadratic terms contribute significantly to the models for almost all responses. The values of R^2 for turbidity and yield were 93.27 and 92.44 respectively. The R^2 value close to 100% suggests that the model fitted to the actual data. Thus the analysis of variance shows that the predicted 2nd order models are statistically valid.

Table 2: Responses for clarified banana juice during RSM optimization studies

Experiment No.	Factors				Responses	
	Enzyme Concentration			Incubation Time	Turbidity	Yield
	Pectinase	Cellulase	Hemicellulase			
	(% w/w)	(% w/w)	(% w/w)	(min)	(NTU)	(%)
1	0.09	0.6	0.35	150	11	75.5
2	0.06	0.4	0.70	60	19	76.1
3	0.09	0.6	0.35	90	15	75.5
4	0.06	0.4	1.40	120	14	76.7
5	0.06	0.4	0.70	120	9	77.7
6	0.03	0.2	1.05	90	20	73.9
7	0.12	0.4	0.70	120	11	77.3
8	0.00	0.4	0.70	120	23	71.0
9	0.06	0.4	0.70	120	10	78.5
10	0.03	0.6	1.05	90	17	75.6
11	0.09	0.6	1.05	90	14	75.5
12	0.09	0.2	1.05	150	12	75.1
13	0.03	0.6	0.35	90	17	74.0
14	0.03	0.2	0.35	90	22	73.2
15	0.06	0.4	0.70	120	9	78.8
16	0.09	0.2	1.05	90	15	75.0

17	0.06	0.0	0.70	120	19	74.2
18	0.09	0.6	1.05	150	10	77.8
19	0.06	0.4	0.70	120	10	79.1
20	0.03	0.2	0.35	150	16	74.1
21	0.09	0.2	0.35	150	12	75.1
22	0.06	0.4	0.70	180	9	76.2
23	0.06	0.4	0.00	120	22	74.1
24	0.03	0.6	0.35	150	14	73.5
25	0.03	0.6	1.05	150	13	74.6
26	0.06	0.8	0.70	120	13	76.3
27	0.06	0.4	0.70	120	8	78.6
28	0.03	0.2	1.05	150	18	73.2
29	0.06	0.4	0.70	120	8	78.7
30	0.09	0.2	0.35	90	16	75.7
31	0.06	0.4	0.70	120	9	78.6

Table 3: Regression Coefficients and R² values for dependent variables for Banana

Term	Regression Coefficient	Turbidity (NTU)	Yield %
Constant	b ₀	9.029*	78.571*
x1	b ₁	-2.358*	1.073*
x2	b ₂	-1.358*	0.456**
x3	b ₃	-0.817***	0.386***
x4	b ₄	-2.058*	0.027
x1.x1	b ₁₁	1.799*	-1.173*
x2.x2	b ₂₂	1.549*	-0.898*
x3.x3	b ₃₃	2.049*	-0.860*
x4.x4	b ₄₄	1.049**	-0.673*
x1.x2	b ₁₂	0.587	0.009
x1.x3	b ₁₃	-0.100	-0.059
x1.x4	b ₁₄	0.038	0.191
x2.x3	b ₂₃	-0.125	0.366
x2.x4	b ₂₄	0.038	0.066
x3.x4	b ₃₄	0.225	0.059
R ²		93.27	92.44

* Significant at 0.001 level

** Significant at 0.01 level

*** Significant at 0.05 level

Table 4: Analysis of Variance (ANOVA) for 2nd Order Model

Source	DF	Turbidity		Juice Yield	
		Adj Sum of	P Value	Adj Sum of	P Value

		Squares		Squares	
Model	14	544.948	<0.001	113.536	<0.001
Linear	4	295.452	<0.001	36.190	<0.001
Square	4	242.709	<0.001	74.435	<0.001
2-Way Interaction	6	6.787	0.827	2.911	0.560
Error	16	39.300	-	9.291	-
Lack-of-Fit	10	35.266	0.028	8.176	0.042
Pure Error	6	4.034	-	1.114	-
Total	30	584.248	-	122.826	-
R ²	-	93.27%	-	92.44%	-
R ² (adj)	-	87.39%	-	85.82%	-

Turbidity

Juice turbidity was significantly ($p \leq 0.05$) affected by the first order and second order of variables (Table 10). From the table it was observed that turbidity has a negative linear effect with all the variables (significant at $p \leq 0.05$) and a positive effect at quadratic level (significant at $p \leq 0.01$). There was no interaction found among the variables for juice turbidity. The effect of experimental variables on juice turbidity is also shown in Fig 2a-2c as response surface and contour plots generated from fitted model. The application of Response Surface Methodology yielded following regression model (after removing non-significant terms), which is empirical relation between response (turbidity) and the test variables in coded units:

$$\begin{aligned} \text{Turbidity (NTU)} = & 9.029^{\dagger} - 2.358 x_1^{\dagger} - 1.358 x_2^{\dagger} \\ & - 0.817 x_3^{++} - 2.058 x_4^{\dagger} + 1.799 x_1^{\dagger} x_1^{\dagger} \\ & + 1.549 x_2^{\dagger} x_2^{\dagger} + 2.049 x_3^{\dagger} x_3^{\dagger} + 1.049 x_4^{\dagger} x_4^{\dagger} \end{aligned}$$

[†] Significant at 0.001 level

⁺⁺ Significant at 0.01 level

⁺⁺⁺ Significant at 0.05 level

Juice Yield

From Table 10 it is clear that juice yield was significantly affected by concentration of enzymes at first order and a positive effect was observed ($p \leq 0.05$). Yield was also found to be effected by all the variables at second order but a negative effect was observed (significant at $p \leq 0.001$). Interaction effects

were not found significant for juice yield also. Influence of enzyme(s) concentration and incubation time on juice yield is shown in Fig 3a-3c as response surface and contour plots generated from fitted model. The following regression model (after removing non-significant terms) was obtained by the application of RSM, which shows empirical relation between response (yield) and the test variables in coded units:

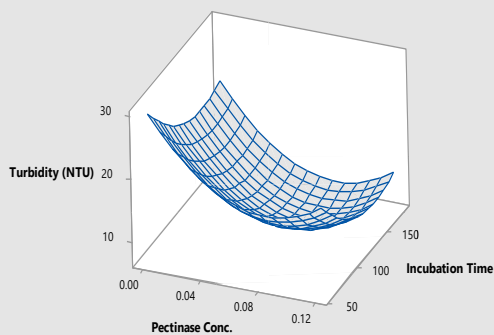
$$\begin{aligned} \text{Yield (\%)} = & 78.571^{\dagger} + 1.073 x_1^{\dagger} + 0.456 x_2^{++} \\ & + 0.386 x_3^{+++} - 1.173 x_1^{\dagger} x_1^{\dagger} - 0.898 x_2^{\dagger} x_2^{\dagger} \\ & - 0.860 x_3^{\dagger} x_3^{\dagger} - 0.673 x_4^{\dagger} x_4^{\dagger} \end{aligned}$$

[†] Significant at 0.001 level

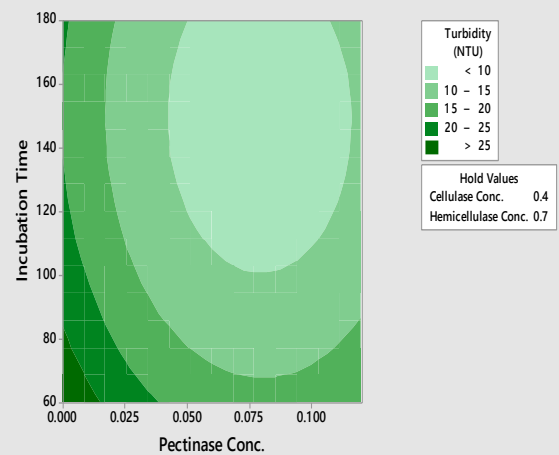
⁺⁺ Significant at 0.01 level

⁺⁺⁺ Significant at 0.05 level

Surface Plot of Turbidity (NTU) vs Incubation Time, Pectinase Conc.

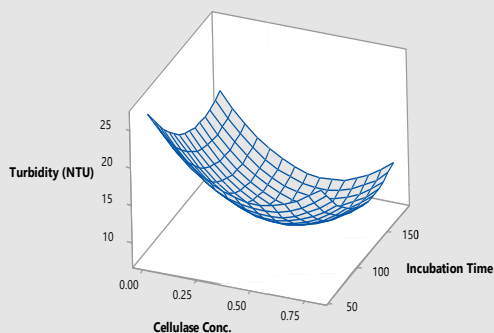


Contour Plot of Turbidity (NTU) vs Incubation Time, Pectinase Conc.

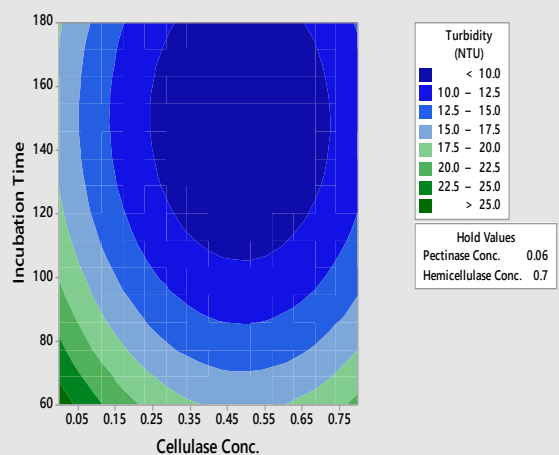


(a)

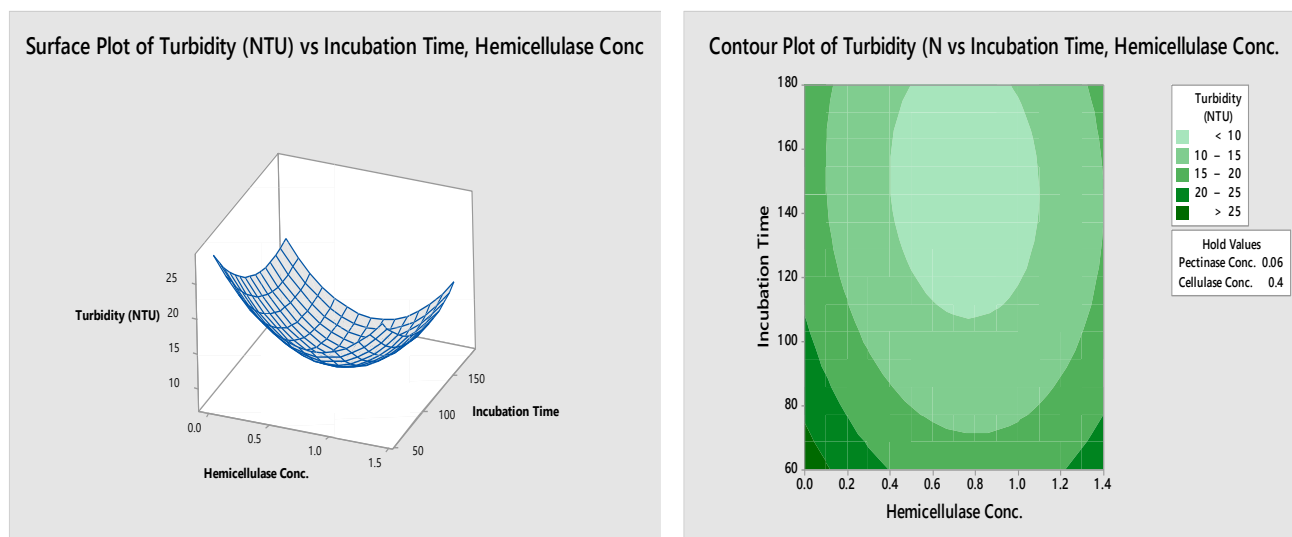
Surface Plot of Turbidity (NTU) vs Incubation Time, Cellulase Conc.



Contour Plot of Turbidity (NTU) vs Incubation Time, Cellulase Conc.

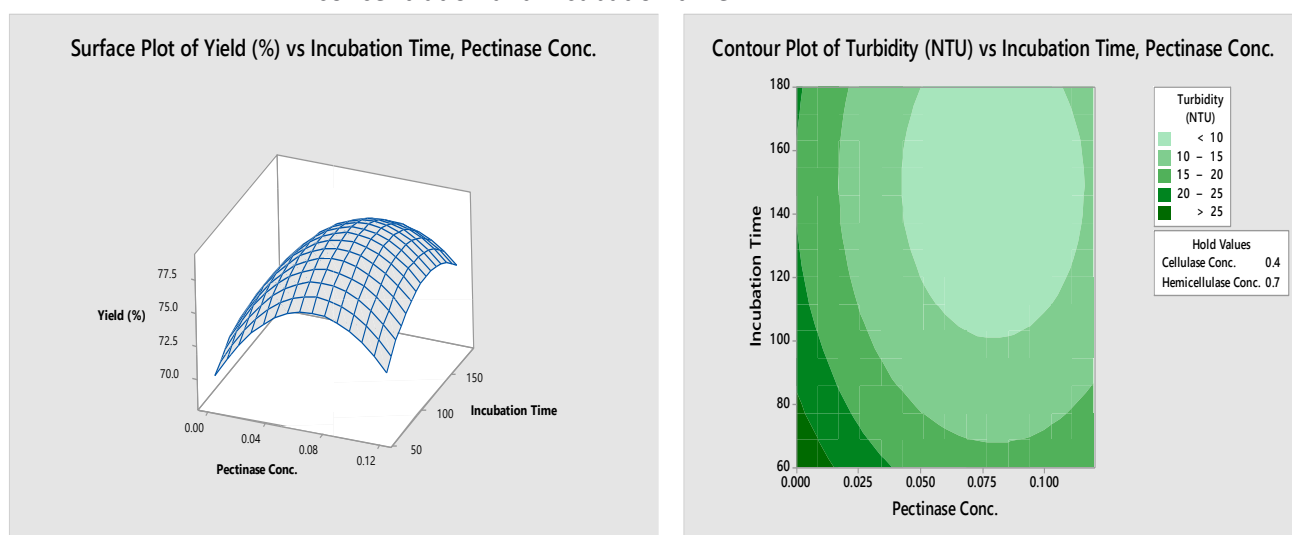


(b)

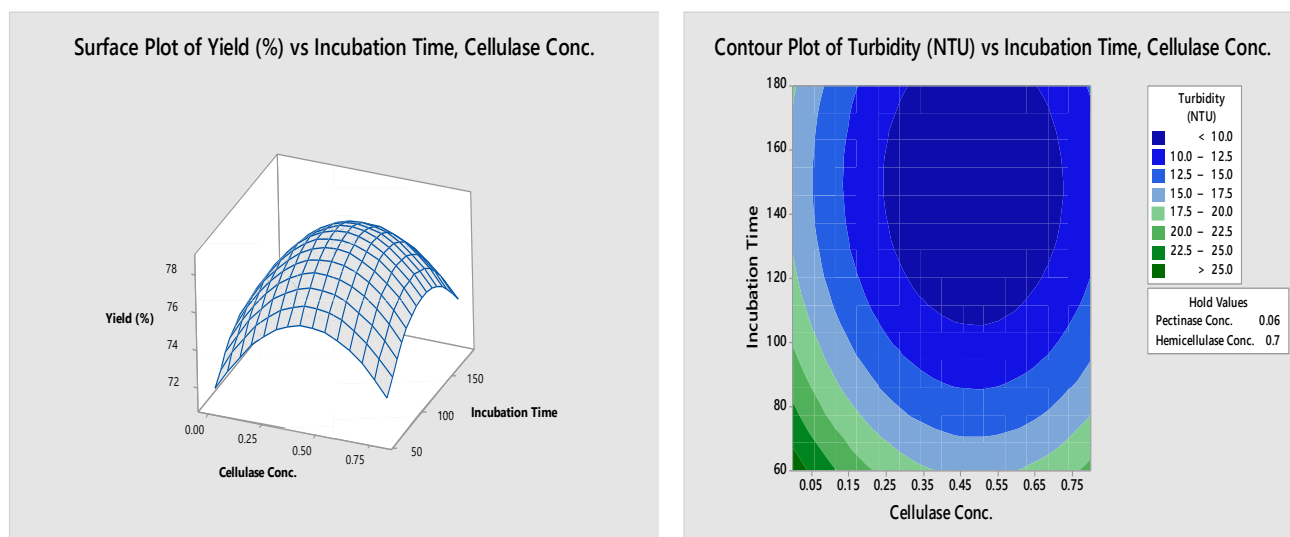


(c)

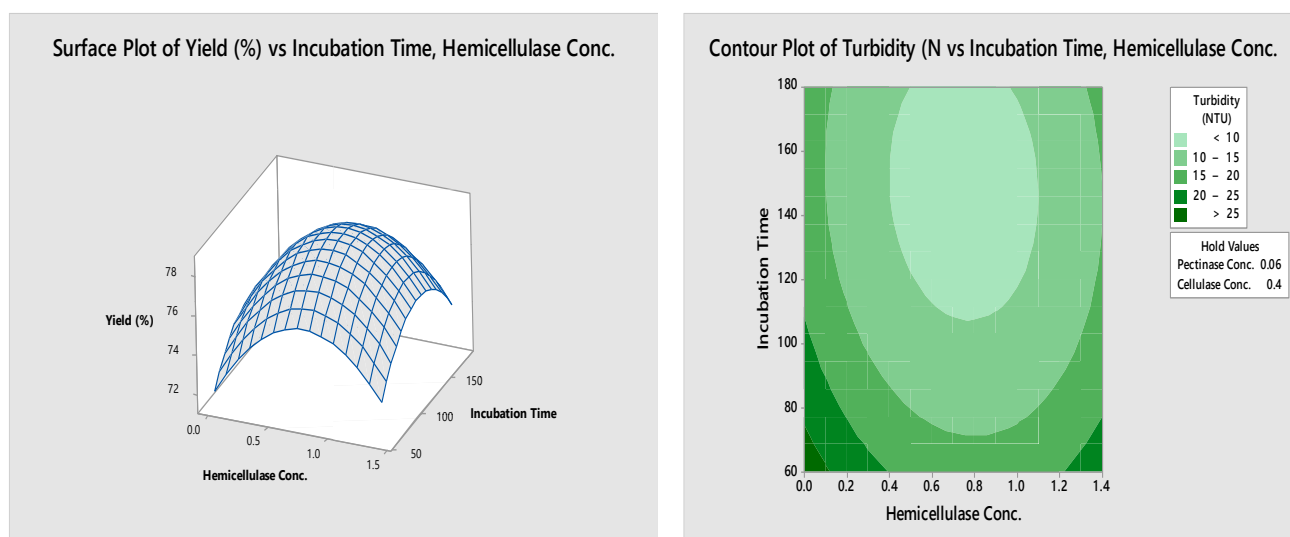
Fig 2 (a,b,c): Surface and Contour plots showing interaction of Juice Turbidity with enzyme (s) concentration and incubation time



(a)



(b)



(c)

Fig 3 (a,b,c): Surface and Contour plots showing interaction of Juice Yield with enzyme concentration and incubation time

Multiple Response Optimizations

For determining the overall optimum conditions in multi-response situation of this study, a two- sided desirability function was used with the responses juice turbidity to be minimized and juice yield to be maximized (Table 5). Similar importance was given to both the responses. Table 6 gives the optimum parameters for multiple response juice turbidity and yield along with the predicted responses which are also shown in optimization plot (Fig 4). The optimization solution for multiple responses juice

turbidity and yield showed treatment of banana juice with 0.07 % (w/w) pectinase, 0.46% (w/w) cellulase and 0.79% (w/w) hemicellulase for 123 min incubation time at 55° C with predicted values of turbidity 7.85 NTU and yield 78.95% and composite desirability as 0.99. When the juice was treated with the optimized set of conditions obtained with response optimizer, the juice turbidity and yield were obtained as 7 and 79.5% which were in good agreement with the predicted values.

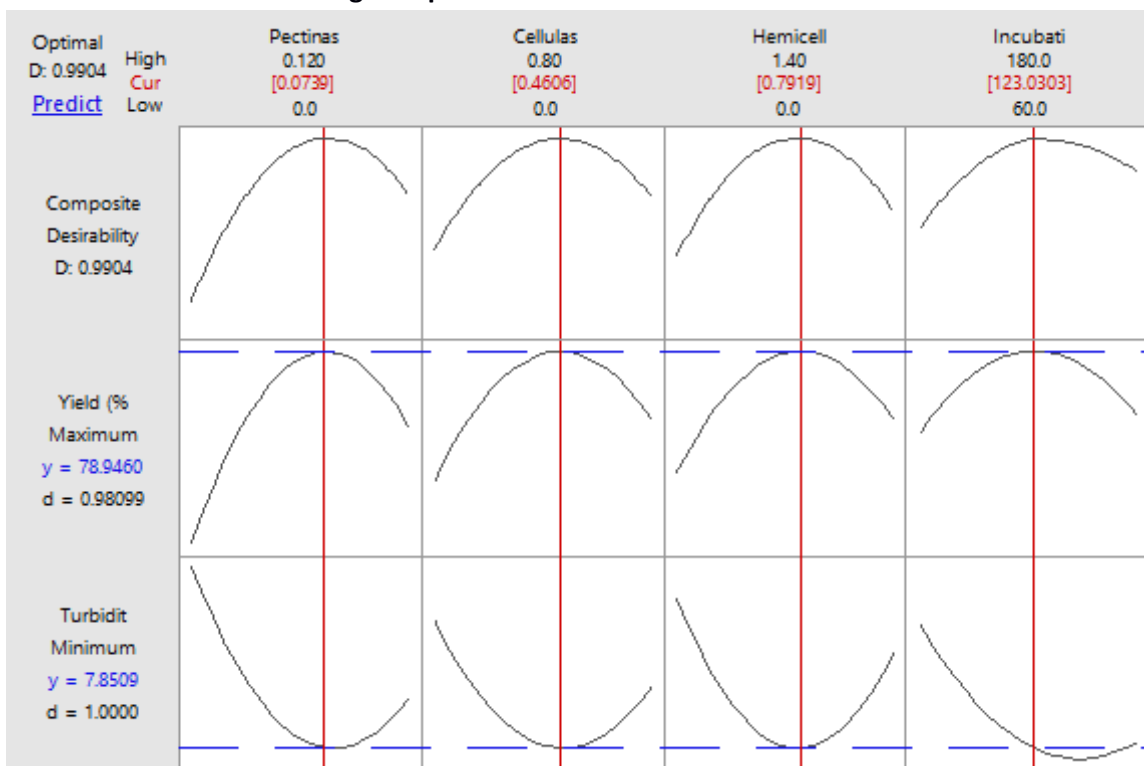
Table 5: Response Optimization Parameters in Response Optimizer:

Response	Goal	Lower	Target	Upper	Weight	Importance
Turbidity (NTU)	Minimum		8.0	23	1	1
Yield (%)	Maximum	71	79.1		1	1

Table 6: Solution (Uncoded Value)

Variables				Multiple Response Prediction		Composite Desirability
Pectinase Concentration	Cellulase Concentration	Hemicellulase Concentration	Incubation Time	Turbidity (NTU)	Yield (%)	
0.0739394	0.460606	0.791919	123.030	7.85093	78.9460	0.990449

Fig. 4: Optimization Plot



Conclusion

The present study showed the effects simultaneous treatment with all three enzymes. The turbidity and % yield improved significantly by simultaneous enzymatic treatment of banana juice. The treatment of banana juice with enzymes was effectively optimized using response surface methodology with a four factor and five level central composite rotatable design which involved thirty-one experiments. Based on Response Surface Optimization, the recommended enzymatic clarification condition for simultaneous treatment of banana juice is 0.07%, 0.46% and 0.79% enzyme concentration for BL-Pectinase, BL-Cellulase and BL-Hemicellulase respectively, at incubation temperature of 55 °C and incubation period of 123 min. Under this condition, the juice was obtained with a very small value of turbidity 7 NTU and 79.5% of yield. Compared to control enzyme-untreated juice, there was 96% decrease in turbidity and 61.3% increase in yield.

References

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