Multienzymatic Clarification Of Totapuri Mango Pulp With The Help Of Response Surface Methodology

Gaurav M. Kunkulol[1], Kalyan P. Babar[2], Balaji Hakke[3], Shrikant S. Bhamare[4]

[1] Research Scholar, Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad, Maharashtra, India
[2] Assistant Professor, Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad, Maharashtra, India
[3] Head Production, NPD at Jain Farm Fresh Foods Ltd, Jain Valley, Shrisoli Road, Jalgaon, Maharashtra India
[4] Manager Quality and R & D, Jain Farm Fresh Foods Ltd, Jain Valley, Shrisoli Road, Jalgaon Maharashtra, India

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Abstract - *Response surface methodology is a statistical method frequently used for optimization studies. Mango juice is viscous and cloudy in nature and contents pectin, starch and xylan. The effect of amylase concentration, pectinase concentration, incubation temperature and incubation time on the juice yield, clarity and colour (L value) of totapuri mango pulp were studied. Totapuri mango juice treated with amylase concentration (0.05-0.45%), pectinase concentration (0.1-0.5%), incubation temperature (40-60⁰C) and incubation time (60-180min). The experimental run for the treatment was designed according to Design expert (10.0) software and the parameters optimized using Response surface methodology. Significant regression models describing the changes of yield, clarity and colour (L value) with respect to the independent variable were established, with R^2 (coefficient of determination) greater than 0.8. Statistical checks (R^2, F value, C. V. and lack to fit test) indicating that the model was adequate for representing the experimental data. From the RSM analysis the optimum processing conditions were found as: 0.15% amylase concentration, 0.20% pectinase concentration, 55⁰C incubation temperature for 150 min time. The recovery (yield), clarity and Colour (L value) of the totapuri mango juice under optimized conditions were 84.24 %, 84.74%T and 54.01 respectively.*

Key Words: Totapuri Mango pulp, Amylase, Pectinase, Response Surface Methodology, Yield, Clarity and Colour (*L* Value).

1.INTRODUCTION

Mango (*Magnifera Indica Linn*) fruit belongs to the family Anacardiaceae is one of the most important commercial fruit crops in India [07]. Mango is indigenous to India; it is cultivated in many tropical and subtropical regions [32]. Mango is king of tropicanol fruits and is well known for its taste, succulence and exotic flavour. Mango is perishable, seasonal fruit which is rich in pectin, sucrose, glucose, maltose, vitamin A, B and C and minerals [06, 30]. Mango also provides a certain amount of minerals and vitamins such as calcium, phosphorous, iron and riboflavin [20]. Mango contains phenols which have powerful antioxidant [36]. Totapuri (Bangalora) mango processed on large scale because this variety has a large yield of pulpy juice with exotic flavour [39].

Mango pulp shows a high ratio of dissolved solid to insoluble fibre, which is responsible for its high viscosity as well as richness in dietary fibre. This fibre consists of pectins, celluloses and starch in addition to free sugars [39]. Mango fruit pulp, 33-85% of the fresh fruit, is essentially composed of protein (0.36-.40 gm), carbohydrate (16.20-17.18 gm), carotene (0.135-1.872 mg), polyphenols, pectin, minerals and other vitamins. Water content of mango pulp more than 80% [25]. Mango pulp is extracted during its seasons and is stored for production of various products [30]. Mango is highly perishable fruit therefore most of fruit processing industry preserves mango pulp for the manufacture of mango pulp product around the year [04].

Juice extraction can be done by using various mechanical processes but in mechanical process yield of juice is low as compare to enzymatic treatment of juice. Mechanical process may be achieved through diffusion, extraction, centrifuge, screw type extractor, decanter, fruit-pulper and by different types of presses [34]. With help mechanical crushing of the pectin rich fruits results in a highly viscous fruit juice from which it is difficult to extract clear juice directly by pressing , due to the fact that mechanical crushing of the tissues give juice that remains bound to the pulp to from a jellified mass [21].

Clarification is a process by which the semistable emulsion of colloidal plant carbohydrates that support the insoluble cloud material of a freshly pressed juice is "broken" [34, 35] such that the viscosity is dropped and the opacity of the cloudy juice is changed to an open splotchy look. This can be accomplished in one of two general ways: enzymatic and non-enzymatically [35]. Presently various methods are used for juice clarification such as enzymatic clarification, centrifugation, ultrafiltration, each filtration and cross flow membrane filtration. Now days enzymatic clarification commonly used in fruit processing industries [35]. In enzymatic clarification process various types of enzyme are used such as pectinase, cellulose, amylase, xylanase etc. [21, 26].

Enzymes are responsible for the degradation of the long and complex molecules in the fruit pulp called as pectin which responsible for turbidity in pulp [42]. Enzymatic degradation of the biomaterial depends on incubation time, enzyme concentration, incubation temperature, agitation, pH and use of different enzyme combination [34]. Use of the different enzyme such as pectinases, amylase, cellulases, alone and their combination can give better juice yield with superior quality of the fruit juice [34].

Pectinase enzyme breakdown complex polysaccharides into simpler molecules like galacturonic acids [17, 27]. In fruit processing industries acidic pectinase used commonly to supress the cloudiness and bitterness of the fruit [17]. For the production of pectinolytic enzyme microorganism such as *Aspergillus niger* or *Aspergillus aculeatus* are widely used [06, 17].

Now days juice industries process edible fruit in large quantities; such fruits are picked while unripe and stored for relatively long periods of time at low temperature. Under this conditions fruit juice contain starch in sufficient amounts to cause turbidity or even gelatinize during juice processing, which makes productive procedures difficult. Therefore demand for amylolytic enzymes, especially glucoamylase has increased in fruit processing industries [21].

Response Surface Methodology is an effective statistical technique for optimization of complex processes. It reduces the number of experiment trial and multiple parameters and their interaction could be studied simultaneously in a single experimental trial [18]. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes [24]. RSM technique has been employed to optimize parameter for enzymatic fruit juice clarification of fruit juice such as Carambola juice [1], Guava juice [03, 33], Carrot juice [05], Blueberry juice [12], Pineapple-Mango blend [14], Carrot-Orange blend [15], Pineapple juice [26], Sapodilla juice [37] and Banana juice [43]. Nowadays RSM is widely used approach to design of several experiments as it decreases number of trials, time and is less laborious than other approaches [14].

Use of the enzyme i.e. cellulose, pectinases, combination of these enzymes and some non-enzyme process can give better quality in terms of clarity of the fruit juice [35]. Enzyme treatment helps in degradation of pectin lead to reduction in water holding capacity of pectin, so that free is release into the system, hence juice recovery increases [35].

Juice clarification is completed by a combination of enzymatic depectinization, gelatin-silica sol, and/or bentonite treatment. The gelatin- silica sol treatment step is particularly slow, mischievous, and requires comprehensive downstream processing to obtain clarified fruit juice [28]. Enzymes are very beneficial to fruit juice industry. Their use results in higher fruit juice yield and improves physical quality characteristics such as clarity, colour, viscosity, filterability etc. [21].

In this study the optimization of combined treatment of Amylase and Pectinase was investigated with respect to temperature, time and maintaining constant enzyme treatment or incubation for clarification of Totapuri Mango pulp by using Response Surface Methodology. The study would have practical application to the mango juice processing industries.

2. MATERIALS AND METHODS

2.1. Materials and processing

2.1.1. Juice samples:

Totapuri mango juice samples were provided by Jain Farm Fresh Foods Ltd, Jalgaon (Maharashtra, India). The control samples of mango pulp were kept at 5^{0}C in aseptic bag used for experimental trials. For each run 100gm of mango pulp was taken.

2.1.2. Enzymatic treatment of juice:

For each experiment 100gm of juice was subjected to different enzyme treatment conditions. The independent processes variable for the enzymatic treatment process were Amylase enzyme concentration (0.05-0.45 %), Pectinase enzyme concentration (0.10-0.50 %), incubation temperature (40-60 ^{0}C) and holding time (60-180 min). The temperature of enzyme treatment was adjusted to the desired temperature using a water bath. At the end of enzymatic treatment, the enzyme in the sample was inactivated by heating the juice at 90^0 C for 5 min in a water bath. After that juice filtered with the help of Muslin cloth then filter juice collected. In clear juice trace amount of Silica sol was mixed and then again filtered with the help of whatman filter paper no 1. Clear juice obtained was evaluated for their clarity, yield and colour.

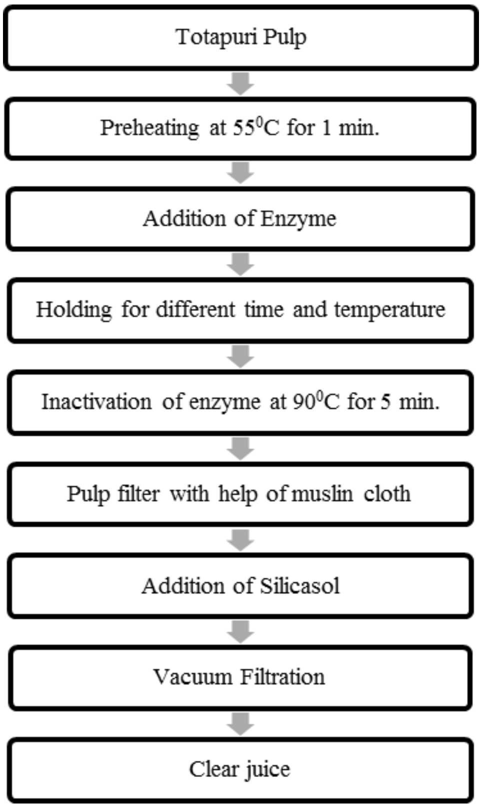


Fig-1: Flow Chart for clarification of juice

2.2. Experimental Design

Design-expert software with central composite design (CCD) was used to determine the effects of four independent variables viz. amylase, pectinase, incubation temperature and incubation time on juice yield, colour and clarity. The range and levels of the variables are given in table. These levels of variables were chosen based on the preliminary experiments. For the design of experiments, the test factors were coded according the following equation:

$$x_i = X_i - X_o / \delta X_i$$

Where, x_i – is the dimensionless coded value of the ith independent variable; X_i – the natural value of the ith independent variable; X_o – the natural value of the ith independent variable at the centre point and δX_i the step change value. After the experiments were performed, the experimental data were fitted with 2nd order polynomial as follows

$$Y=b_0+b_1x_1+b_2x_2+b_3x_3+b_4x_4+b_{11}x_1^2+ b_{22}x_2^2+ b_{33}x_3^2+ b_{44}x_4^2+ b_{12}x_1x_{2+} b_{13}x_1x_{3+} b_{14}x_1x_{4+} b_{23}x_2x_{3+} b_{24}x_2x_{4+} b_{34}x_3x_4$$

Where, Y is the predicated response; b_o the intercept; b_1, b_2, b_3, b_4 the linear co-efficient; b_{11}, b_{22}, b_{33}, b_{44} the squared co-efficient and b_{12}, b_{13}, b_{14}, b_{23}, b_{34} the interaction co-efficients.

Table - 1: The experimental domain

Independent Variable	Coded Variable				
	-2	**-1**	**0**	**1**	**2**
Amylase (%)	0.05	0.15	0.25	0.35	0.45
Pectinase (%)	0.1	0.2	0.3	0.4	0.5
Temp. (^{0}C)	40	45	50	55	60
Time (min)	60	90	120	150	180

2.3. Response analysis

2.3.1. Yield

The clear Juice yield estimated as a percentage of weight of the clear juice obtained to the initial puree. The formula was

$$\%\ \text{Yield} = \frac{\text{Weight of Clear Juice}}{\text{Weight of Initial Pulp}} \times 100$$

2.3.2. Clarity

Clarity was determined by measuring the transmittance at 625nm using a UV-Visible spectrophotometer (Model Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA). Distilled water was used as the reference.

2.3.3. Colour measurement

The Colour of the clarified juice was measured with help of Hunter Laboratory Calorimeter (Model Colour Flex Ez spectrophotometer, Hunter Laboratory, Inc., Virginia). Where *'+L'* Value represents lightness and *'–L'* represents darkness.

2.4. Data analysis

All statistical experimental designs and results analysis were carried out by using Design- Expert (Trial Version 10; STAT-EASE Inc., Minneapolis, MN, USA) software. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2, and its statistical significance checked by Fisher's *F*-test. The significance level of each regression co-efficient was determined by student's *t*-test. The level of significance was given as *p*-value.

2.5. Optimization Strategy

The process was numerically optimized with respect to 'minimum' amylase and pectinase and 'in range' incubation temperature and incubation time. The goal for yield, clarity and *L* value were taken as maximum.

Table - 2: The experimental designs and results of totapuri mango clarified juice.

Run	Coded Level				Observed Responses		
	A	B	C	D	Yield (%)	Clarity (%)	*L* Value
1	-1	-1	-1	-1	79.8	70.88	50.94
2	1	-1	-1	-1	80.23	76.74	52.22
3	-1	1	-1	-1	78.23	75.75	51.42
4	1	1	-1	-1	80.4	77.27	52.79
5	-1	-1	1	-1	84.7	76.29	52.14
6	1	-1	1	-1	83.29	87.03	54.44
7	-1	1	1	-1	79.12	87.16	54.45
8	1	1	1	-1	89.35	87.88	54.42
9	-1	-1	-1	1	84.35	78.48	52.66
10	1	-1	-1	1	85.45	88.12	54.44
11	-1	1	-1	1	83.12	81.79	52.89
12	1	1	-1	1	90.35	84.84	53.29
13	-1	-1	1	1	84.48	85.24	54.08
14	1	-1	1	1	84.38	89.29	54.48
15	-1	1	1	1	87.43	93.54	56.88
16	1	1	1	1	91.41	85.18	53.18
17	-2	0	0	0	78.16	76.02	51.45
18	2	0	0	0	84.2	87.86	54.3
19	0	-2	0	0	82.14	78.5	52.52
20	0	2	0	0	89.42	84.58	53.24
21	0	0	-2	0	80.09	76.65	51.9
22	0	0	2	0	86.44	92.01	55.17
23	0	0	0	-2	79.3	73.89	51.1
24	0	0	0	2	88.22	84.88	53.13
25	0	0	0	0	82.24	74	51.15
26	0	0	0	0	82.4	73.9	51.01
27	0	0	0	0	82.12	74.2	51.28
28	0	0	0	0	82.28	73.95	51.08
29	0	0	0	0	82.08	74.15	51.24
30	0	0	0	0	82.42	74.12	51.21

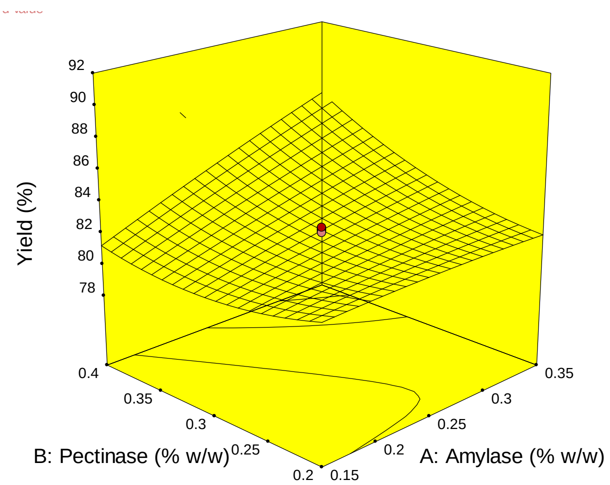
3. RESULTS

Recently enzymes have been extensively used in fruit juice clarification industries because of some advantages such as (a) complete degradation of polysaccharide into simple soluble sugar (b) maximum juice clarification (c) increases juice yield with natural colour, aroma and phenolic compound [34]. The clarified juice extracted from enzyme treated and untreated (control) clarified juice was evaluated for juice yield (%), clarity (% T) and *L* value (colour). Table no. 2 shows the juice yield clarity and colour under the different experimental condition enzyme combination. Response Surface Methodology is widely used method for the optimization study of the experimental and it is helps in decreasing number of trials. Therefore this technique was applied in this study. According to central composite design 30 trials were conducted. All the trials conducted by the design of

Table - 3: Co-efficient of the regression equation for totapuri mango clarified juice.

Term	Yield		Clarity		Colour	
	Co-efficent	*p*-value	Co-efficent	*p*-value	Co-efficent	*p*-value
Intercept	82.26	<0.0001	74.05	<0.0001	51.16	<0.0001

A-Amylase	1.49	0.0001	2.12	<0.0001	0.4	0.0127
B-Pectinase	1.14	0.0013	1.4	0.0024	0.22	0.1314
C-Temperature	1.46	0.0001	3.69	<0.0001	0.83	<0.0001
D-Time	2.24	<0.0001	2.89	<0.0001	0.55	0.0014
A^2	-0.16	0.5528	2.15	<0.0001	0.52	0.0012
B^2	0.99	0.0023	2.05	<0.0001	0.52	0.0011
C^2	0.36	0.2036	2.74	<0.0001	0.69	<0.0001
D^2	0.48	0.0938	1.51	0.0008	0.33	0.0224
A×B	1.47	0.0008	-2.08	0.0005	-0.48	0.0131
A×C	0.11	0.7579	-0.81	0.1055	-0.37	0.0495
A×D	0.049	0.8904	-0.65	0.1834	-0.38	0.0437
B×C	0.51	0.1669	0.66	0.1827	0.23	0.2019
B×D	0.91	0.0207	-0.81	0.106	-0.17	0.3302
C×D	-0.84	0.0315	-1.11	0.0323	-0.17	0.3336



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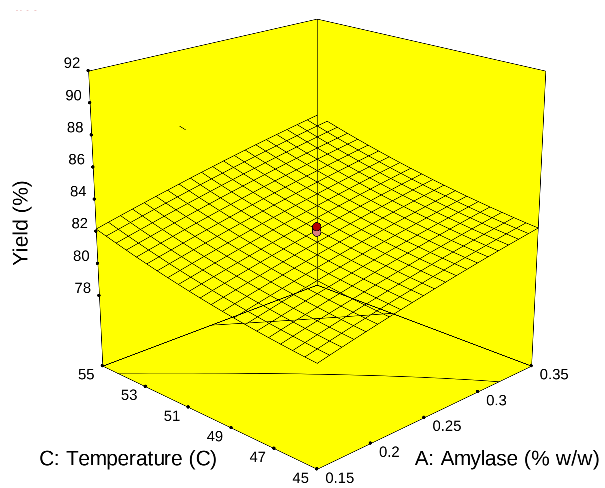

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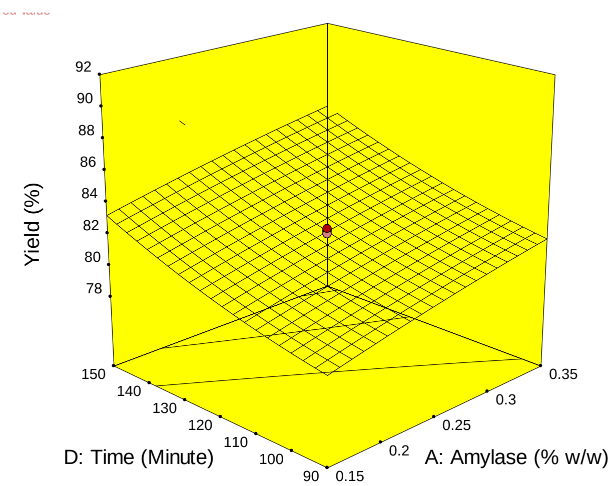

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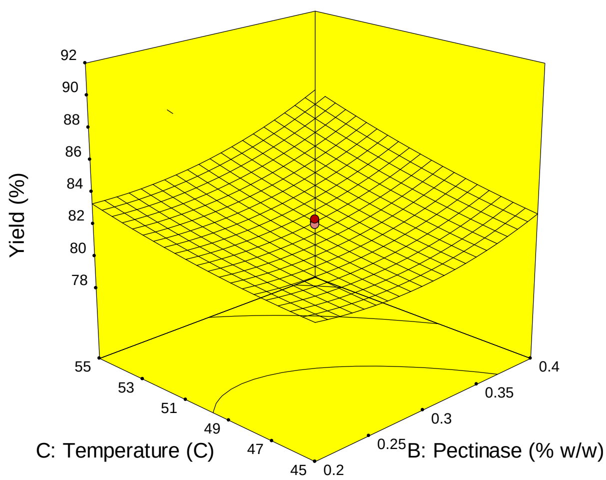

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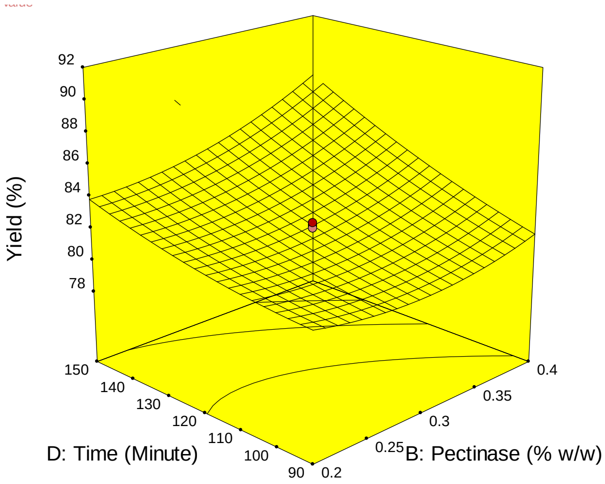

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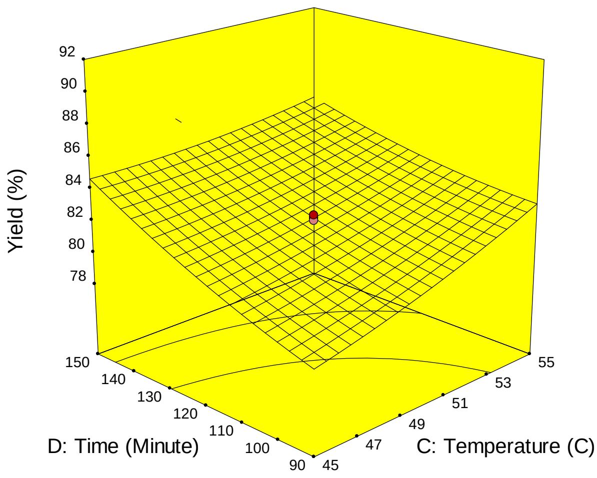
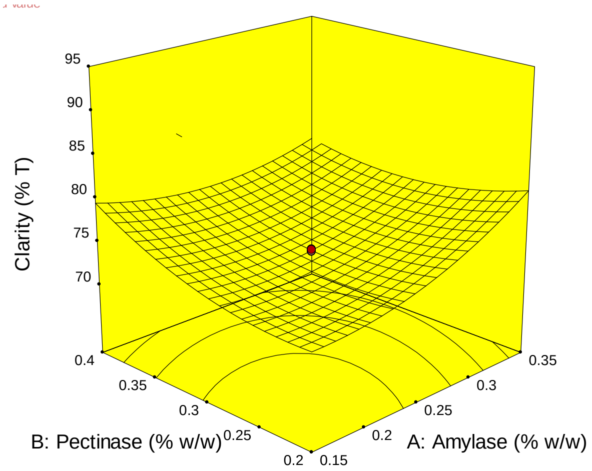


(f)

Chart-1: Three dimension plot for yield of totapuri mango clarified juice as function of (a) amylase and pectinase ; (b) amylase and incubation temperature ; (C) amylase and incubation time; (d) pectinase and incubation temperature ; (e) pectinase and incubation time; (f) incubation temperature and incubation time.



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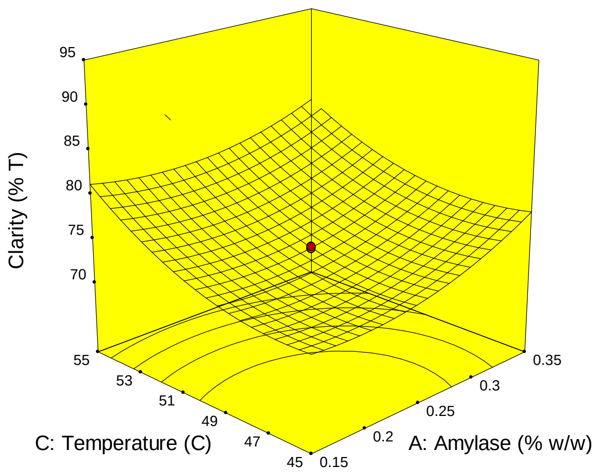

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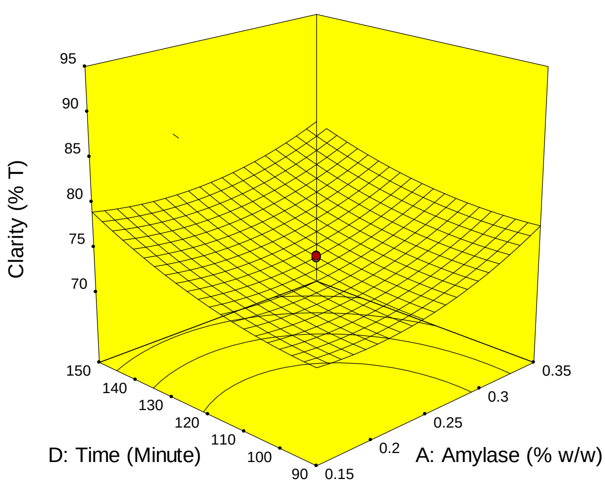

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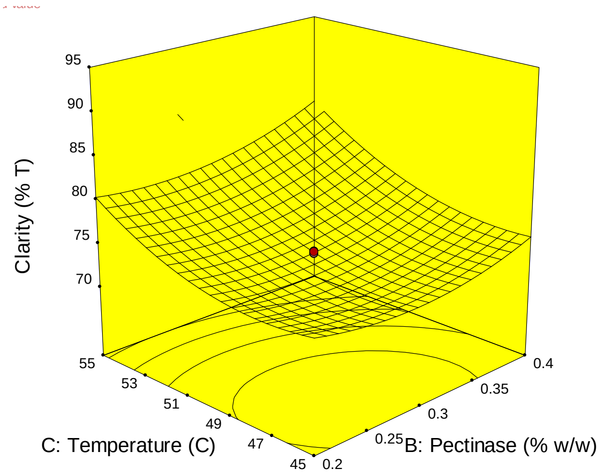

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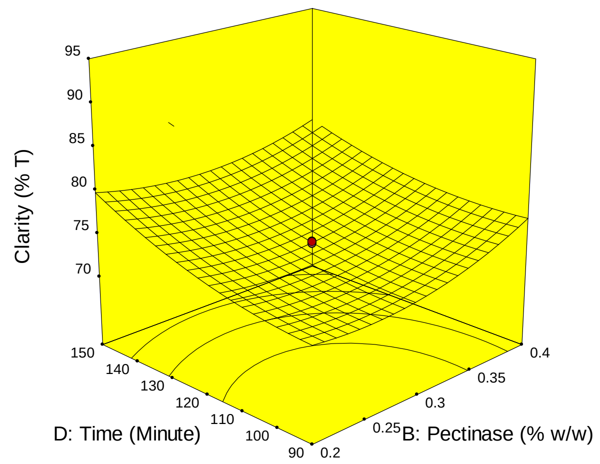

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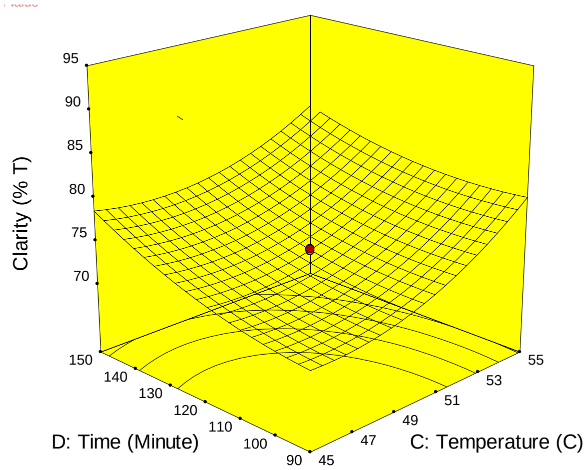
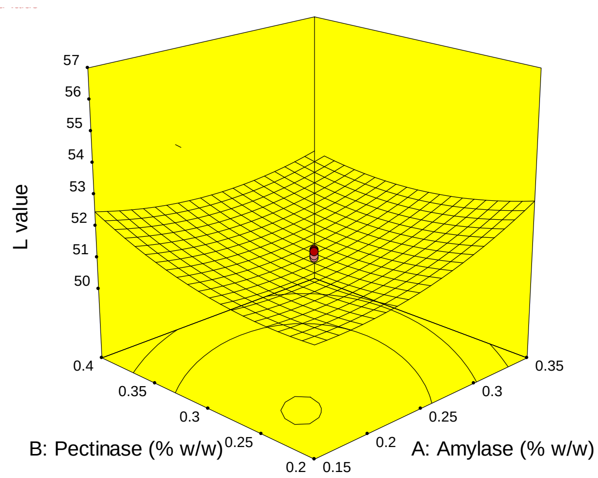


(f)

Chart-2: Three dimension plot for clarity of totapuri mango clarified juice as function of (a) amylase and pectinase ; (b) amylase and incubation temperature ; (C) amylase and incubation time; (d) pectinase and incubation temperature ; (e) pectinase and incubation time; (f) incubation temperature and incubation time .



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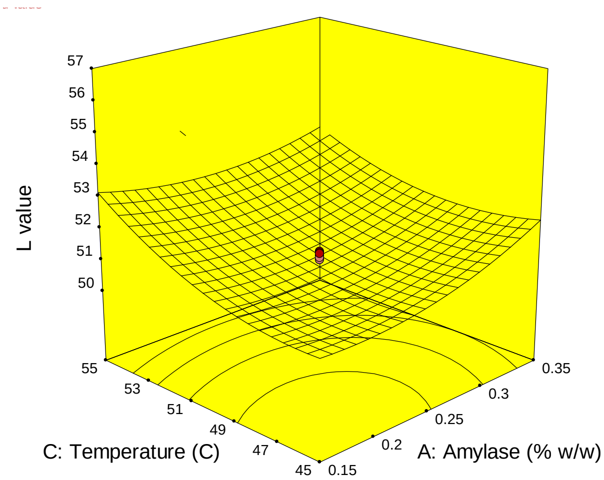

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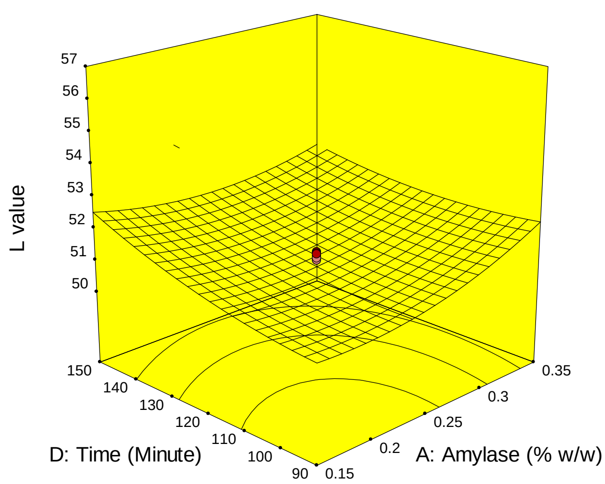

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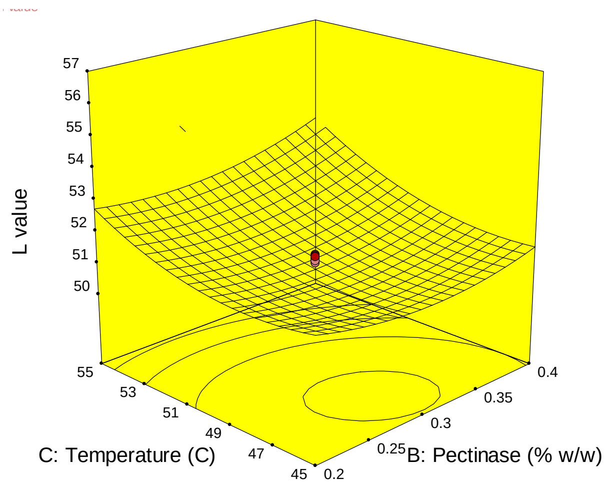

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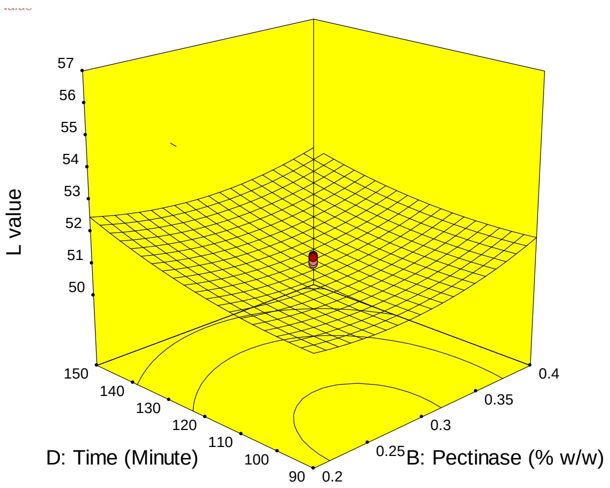

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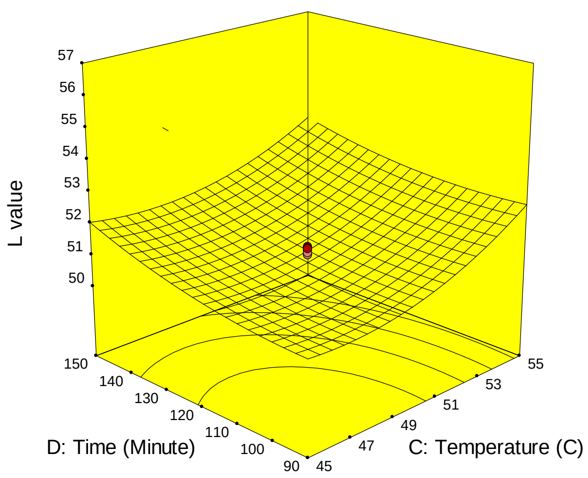


(f)

Chart-3: Three dimension plot for colour (*L* value) of totapuri mango clarified juice as function of (a) amylase and pectinase ; (b) amylase and incubation temperature ; (C) amylase and incubation time; (d) pectinase and incubation temperature ; (e) pectinase and incubation time; (f) incubation temperature and incubation time.

experiment. The experimental run values for all three responses such as juice yield, clarity and colour (*L* value) under different condition are given in table no. 2.

The effects of change in variable on process response are explained in turns of their statistical coefficient in table no. 3. The analysis of variance (ANOVA) of three independent variables i. e, yield, clarity and colour, showed the experiment data add coefficient of determination near to unity with the calculated model no significant lack to fit at < 0.05. The variable were analyzed for their linear, quadratic and interactive effect gave the following equation (in terms of coded unit) to predict the juice yield, clarity and colour with experimental domain.

Juice yield = 82.26 + 1.49 (Amylase) + 1.14 (Pectinase) + 1.46 (Temperature) + 2.24 (Time) 1.47 (Amylase × Pectinase) + 0.11 (Amylase × Temperature) + 0.049 (Amylase ×Time) + 0.51 (Pectinase × Temperature) + 0.91 (Pectinase × Time) – 0.84 (Temperature × Time) - 0.16 (Amylase^2) + 0.99 (Pectinase^2) + 0.36 (Temperature^2) + 0.48 (Time^2)

Juice clarity = 74.05 + 2.12 (Amylase) + 1.40 (Pectinase) + 3.69 (Temperature) + 2.89 (Time) -2.08 (Amylase × Pectinase) – 0.81 (Amylase × Temperature) -0.65 (Amylase ×Time) + 0.66 (Pectinase × Temperature) -0.81 (Pectinase × Time) – 1.11 (Temperature × Time) + 2.15 (Amylase^2) + 2.05 (Pectinase^2) + 2.74 (Temperature^2) + 1.51 (Time^2)

Juice colour = 51.16 + 0.40 (Amylase) + 0.22 (Pectinase) + 0.83 (Temperature) + 0.55 (Time) -0.48 (Amylase × Pectinase) – 0.37 (Amylase × Temperature) -0.38 (Amylase ×Time) + 0.23 (Pectinase × Temperature) -0.17 (Pectinase × Time) – 0.17 (Temperature × Time) + 0.52 (Amylase^2) + 0.52 (Pectinase^2) + 0.69 (Temperature^2) + 0.33 (Time^2)

Table - 4: Regression analysis (ANOVA) for process response of totapuri mango clarified juice.

Response	Source	Sum of square	Degree of Freedom	Mean square	F-value	P-value
Yield	**Model**	335.43	14	25.25	12.71	<0.0001
	Residual	29.8	15	1.99		
	Total	383.24	29			
	C. V.=1.69%		R^2=0.9222		Adj R^2= 0.8496	
Clarity	**Model**	1172.12	14	83.72	23.81	<0.0001
	Residual	52.74	15	3.52		
	Total	1224.86	29			
	C. V.=2.32%		R^2=0.9569		Adj R^2= 0.9168	
Colour	**Model**	61.25	14	4.38	9.31	<0.0001
	Residual	7.05	15	0.47		
	Total	68.3	29			
	C. V.=1.30%		R^2=0.8968		Adj R^2= 0.8004	

3.1. ANOVA

The model was judged for its adequacy by the Fisher's *F*-test. If the model is good predictor of the experiment results that time calculated *F*-value should be several times greater than p value as per software. *F* value obtained 12.71 for yield, 23.81 for clarity and 9.31 for colour. Therefore pass Fisher's F-test. The acceptability of model was also verified by very low probability value (p model < 0.001) for all the responses and there is a quadratic relationship between the independent variable and response variable. The goodness of fit model was examined by the coefficient of determination (R^2) is defined as the ratio of the total variation and is a measure of the degree of fit. The closer the value of R^2 value of unity, the better empirical model fits the actual data. The R^2 in the model were (0.9222, 0.9569 and 0.8968). Therefor R^2 values for the response variable were than higher than 0.8 which indicates the empirical model fits the actual data. The coefficient of variation (C.V.) describes to which extent the data are dispersed and C. V. defined as a measure mean. The lower values of coefficient of Variation (C.V. yield = 1.69 %, C. V. clarity = 2.32 % and C. V. Colour= 1.30 %) suggested that the experimental results were precise and reliable [14, 26, 31, 38].

3.2 Response Surface Analysis

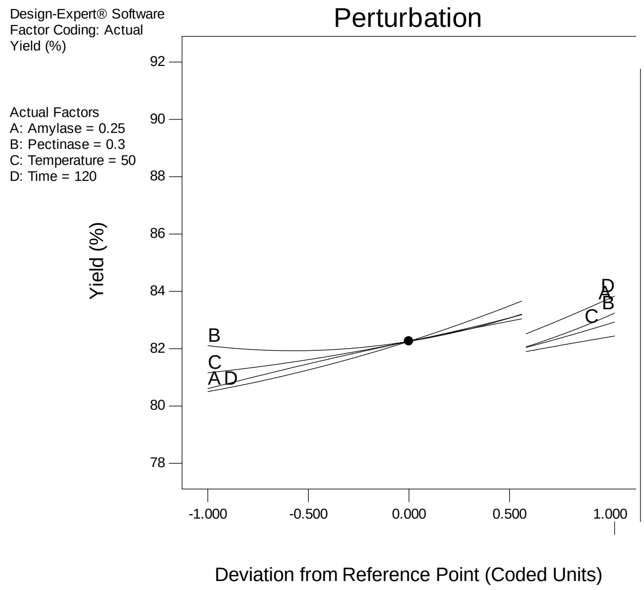
3.2.1 Juice Yield

Pectinase helps in pectin hydrolysis, which causes reduction in pulp viscosity and a significant increase in clarified juice Yield [06]. The juices obtained after multienzymatic treatment had more yield and corresponding clarity than untreated one i. e. control because of the reduction of xylan, pectin and starch content [06]. For the first composition yield of the extracted juice was in a range from 78.16 to 91.41 %. The minimum yield was observed when concentration amylase 0.05 %, concentration pectinase 0.30 % was used for 120 min at 50°C. The maximum yield was at all coded variable '+1' means amylase 0.35 %, pectinase concentration 0.40 % at 55°C for 150 min.

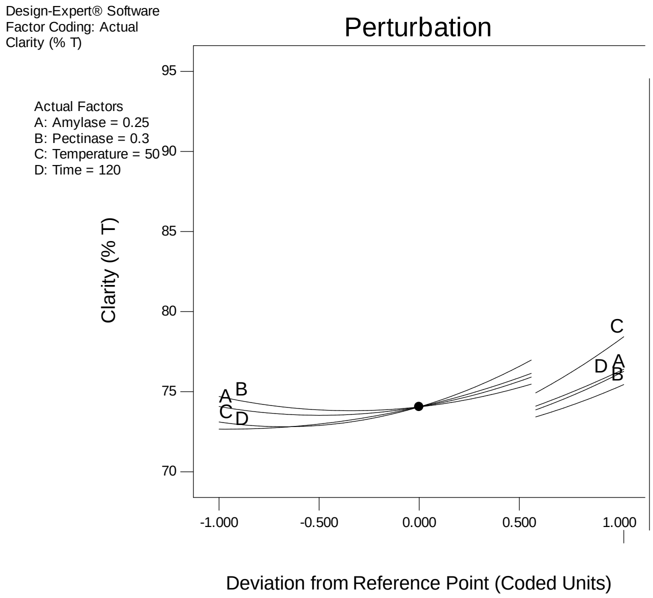
All the independent variables with respect to their importance were checked by keeping values changing of two independent variables and remaining two independent were kept constant. Chart 1 showing the three dimensional response surfaces plot for the effect of the independent variable on the yield of clarified juice. To understand the interaction of different variables and the optimum level of each variable the response surfaces curves were plotted. Each response surface curves explains the effect of two variables while remaining two variables at middle level. In Chart 1 (a) represents the interactive effect of Amylase (%) and Pectinase (%) on the juice yield, whereas the temperature (^{0}C) and time (min) was kept at middle level that is 50^{0}C temperature for 120 min. Similarly all independent variables interchanged by keeping two constant and two variables. Chart 1 (b) shows interactive effect between temperature (^{0}C) and Amylase (%) where other variables i.e. Pectinase (%) and time (min) was kept constant.

3.2.2 Clarity

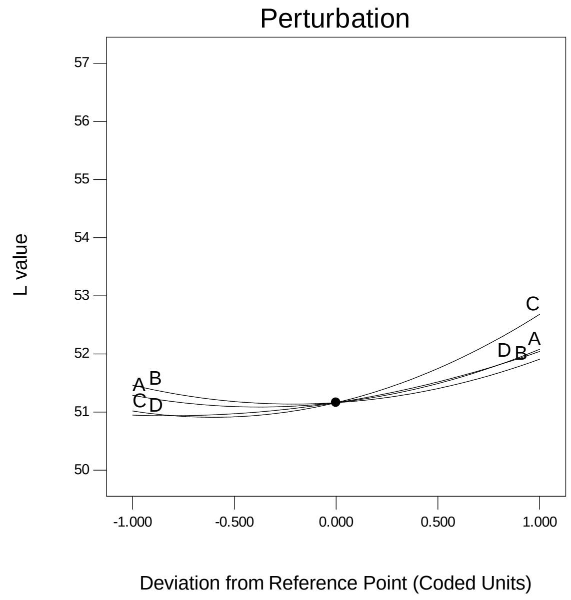
The clarity was an important parameter of clarified juice [41]. Clarified juice clarity ranged from 70.88 % T to



(a)



(b)



(c)

Chart-4: Perturbation graph showing the effect of independent variables on (a) yield (b) clarity and (C) colour of totaouri mango clarified juice (A- amylase, B- pectinase, C- incubation temperature, D- incubation time).

Table - 5: Constraints, criteria for optimization, solution, along with predicted and observed response value of mango clarified Juice.

Criteria	Goal	Importance	Solutions	Observed Response
Amylase (% w/w)	Minimize	3	0.15	
Pectinase (% w/w)	Minimize	3	0.2	
Incubation Temperature (^{0}C)	In the range	3	55	
Incubation Time (Min)	In the range	3	150	
Yield (%)	Maximize	3	84.04	84.24
Clarity (%T)	Maximize	3	83.97	84.74
L Value	Maximize	3	54.02	54.01

93.54 % T (table no. 1). The clarity was maximum in run no. 15 when amylase concentration 0.15 %, pectinase concentration 0.40 % used for 150 min at 55^{o}C, whereas minimum juice clarity was at amylase concentration 0.15 %, pectinase concentration 0.20 % at 45^{o}C for 90 min. All the four variables with respect to their importance were checked by keeping values changing of two independent variables and remaining two independent were kept constant. Chart 2 (a) represents the 3-D surface graphics showing the interactive effects between variables pectinase and amylase where the variable kept constant at 50^{0}C temperature and time 120 min respectively. Similarly all graphs were represented.

3.2.3 Colour (*L* value)

The appearance characteristics, for example colour i. e. lightness or darkness was the first judgement of a clarified juice quality. A dark product would mean that the clarified juice was deteriorating and it was usually less appealing to the consumer [41]. A dark product means that the clarified juice is deteriorated and it is usually less appealing to the consumer [18]. *L* value of the Mango clarified juice ranges from 50.94 to 56.88. The *L* value 50.94 showing the product was dark therefore consumer will not prefer to buy it. Chart 3 (a) represent the interaction effect of amylase and pectinase on colour. Likewise all graphs were represented.

3.3 Perturbation Interpretation

The simultaneous effect of variation in levels of all the four independent factors on process response can be seen in perturbation graph (Chart 4). Curve indicates yield (%), clarity (%T) and colour (*L* value) in Chart 4. According to the curve shown in Chart 4 (a), as the incubation time and pectinase concentration increases, yield of juice also increases. This is due to the presence of pectin compound in the mango juice which affects the yield of clarified mango juice. Clarity (% T) and colour (*L* value) of clarified juice depends on the incubation temperature. Perturbation curve indicates enzyme activity depends on the incubation temperature therefore it is necessary to maintain the optimum temperature in the fruit juice clarification process.

3.4 Optimization

Even though the purpose of using enzyme in fruit processing process is to improve physio chemical characteristic of the product, cost also is a crucial industrial factor to be taken consider into account in order to achieve a reasonable operational condition, cost on enzyme is more therefore enzyme concentration is the one which has the greatest impact on the process cost. Thus low enzyme concentration was used to optimization condition. The optimization condition for the production of mango clarified juice containing maximum yield, colour and clarity was determined by the numerical optimization with chosen each variable and response was given table no. 5.
For this composition the predicted that conditions which contain amylase concentration 0.15 %, pectinase 0.2 % at 55^{0}C temperature for 150 min incubation time would produce the maximum values clarified mango juice yield 84.04 %, with clarity 83.97 %T and *L* value 54.02.
The suitability of the modern equation was performed using the recommended optimum condition. The experimental result shows in the table no. 5. This result shows that closer to the predicted values by the software which indicating that each model was quite accurate in prediction.

4. CONCLUSION

Central Composite design was found to be efficient and valuable statistical tool for analysing and optimizing the effects of enzyme concentration, incubation temperature and incubation time on multienzymatic clarification of mango juice clarification. The recommended enzymatic treatment for composition is pectinase 0.15%, amylase 0.20%, incubation temperature 55^{0}C at for 150min. The optimum condition of the model equation was performed using the recommended optimal conditions shows that close to predicate values indicating that each model was quite accurate in prediction.

This study would be helpful for fruit processing industries worldwide, especially this study helpful for mango processing industry as the potential procedure of mangoes juice and clarified mango juice because of the good demand and for its known nutritive value and taste. The response surface and numerically optimization methods give rise to a better understanding the optimizing the clarification process.

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