



# Optimization of Prickly Pear Juice Clarification for Better Retention of Betalain and Color Properties

P.R. Davara, A.K. Varshney, V.P. Sangani, P.P. Vora

10.18805/ajdfr.DR-1693

## ABSTRACT

**Background:** Prickly pear juices are considered as valuable ingredient for sports and energy drinks due to its higher amino acids contents, minerals and attractive red color. Hence, prickly pear fruit has received renewed interest for the production of juice. Up to date, relatively little work has been reported regarding the manufacturing of prickly pear products. Enzyme has proved to be the key element for producing clear and stable fruit juice. The present study aimed to optimize the processing parameters for enzymatic clarification of prickly pear juice. The results of this investigation are expected to provide the suitable process technology for the production of prickly pear juice with better retention of betalain content and color value.

**Methods:** Prickly pear pulp was treated with pectinase enzyme at different concentrations (0.01-0.09%), incubation temperatures (40-60°C) and incubation time (60-180 min). The effect of enzymatic treatments on clarity, color index, betalain content and ascorbic acid content of juice were studied through response surface methodology by employing second order central composite rotatable design.

**Result:** Response surface analysis determined the optimum condition for clarifying prickly pear juice as 0.036% enzyme concentration, 46°C incubation temperature and 112 min incubation time. At this condition, it was predicted to produce the prickly pear juice with clarity of 48.59% T, color index of 0.603 abs, betalain content of 542.93 mg/L of juice and ascorbic acid content of 9.35 mg/100 g with the desirability of 0.780.

**Key words:** Betalain content, Color, Enzymatic treatment, Juice clarification, Pectinase, Prickly pear.

## INTRODUCTION

Juice production is one of the most frequently utilized process technologies in the fruit processing industry. Juices are much appreciated for their nutritive value and sensory properties (Kgatla *et al.*, 2011). Prickly pear juices are considered as a valuable ingredient for sports and energy drinks due to its higher amino acids contents (Seidl *et al.*, 2000). The pulp has an attractive color that varies from soft green, greenish-white, canary-yellow, orange-yellow, lemon-yellow, red and cherry-red to purple hues (Sáenz, C. and Sepúlveda 2001). These attractive colors are due to the betalains comprising the red-violet betacyanins and the yellow-orange betaxanthins (Fernandez- López and Luis-Almela 2001). The other functional benefits such as immunity boost up, bones and teeth building, digestive health maintenance, heart health enhancement, anti-carcinogenic effects, antioxidant potential, weight loss effects and inflammation suppression are also associated with this fruit (Joshi *et al.*, 2020). Due to this color property, prickly pear juice has been considered as valuable source for enhancing the nutritional content and color in fruit juice blends *viz.*, orange-apple juice blends (Moreno- Álvarez *et al.*, 2003). The key of producing clear and stable fruit juice is a complete enzymatic breakdown of pectin (Lee *et al.*, 2006). The treatment of mash with pectinases causes the degradation of cell wall and middle lamella of plant cells. This releases the fruit juice and increases yield (Demir *et al.*, 2001; Saxena *et al.*, 2014). (Enzymatic treatments using pectinase have also assisted in the improvement of yield and reduction of

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**How to cite this article:** Davara, P.R., Varshney, A.K., Sangani, V.P., Vora, P.P. (2022). Optimization of Prickly Pear Juice Clarification for Better Retention of Betalain and Color Properties DOI: 10.18805/ajdfr.DR-1693.

**Submitted:** 19-04-2021 **Accepted:** 16-10-2021 **Online:** 07-01-2022

turbidity of prickly pear juice (Davara *et al.*, 2017). The fundamental physical property of food products is color. Color of juice is affected by natural enzymes, oxidation of ascorbic acid and the Maillard reaction, which depends on the content of reducing sugars, proteins and temperature (Mackay *et al.*, 1990). The enzymatic hydrolysis of pectic substances depends on several processing variables such as type of enzyme, hydrolysis time, enzyme concentration, incubation temperature and pH (Lee *et al.*, 2006; Sin *et al.*, 2006). These parameters need to be optimized for maximum clarity and color retention of juice. In order to this, optimization studies for prickly pear juice clarification were conducted using response surface methodology (RSM). The aim of the study was to assess the effect of enzymatic process parameters on different quality and color characteristics of clarified

prickly pear juice and to optimize the process condition through response surface methodology.

## MATERIALS AND METHODS

The experiment was carried out at Dept. of Processing and Food Engineering, College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh.

### Raw materials

The prickly pear fruits were collected from the plants grown at nearby areas of Junagadh (Gujarat, India). Fully ripe fresh, bright red and purple color fruits, without any visual defects, were selected for the experimental work. Commercial food grade pectinase enzyme was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai (Maharashtra, India) and stored at 4°C. The activity of pectinase enzyme as reported by the supplier was 8000-12000 U/g.

### Enzymatic clarification

For each experiment, 100 g pulp was subjected to different enzymatic treatments under different conditions as shown in Table 1. The pectinase enzyme was added to each pulp sample and placed in an incubator for incubation at desired levels of temperature and time. At the end, suspension was heated in a water-bath at 90°C for 5 min to inactivate the enzyme present in the sample (Lee *et al.*, 2006). The enzyme treated prickly pear pulp samples were then centrifuged at 3600 rpm for 10 minutes. The supernatant was collected and then filtered using 2 folds muslin cloth to get the clear prickly pear juice. It was then filled in glass bottles and stored at 7 °C in refrigerator for further analysis.

### Physicochemical analysis

Clarity was determined by measuring the percent transmittance (%T) at wavelength of 660 nm using UV-VIS spectrophotometer (UV 5704SS, Electronics Corporation of India Pvt. Ltd., Hyderabad, India) (Lee *et al.*, 2006). Distilled water was used as reference. The color index was determined as per the method proposed by Meydov *et al.* (1977). The betalain content of prickly pear juice was quantified as per the method proposed by Nilsson (1970). The betalain content was calculated using an equation proposed by Cai and Corke (1999). Ascorbic acid was determined by the method described by Hans (1992). The ascorbic acid content was calculated as mg/100 g of edible portion.

### Experimental design and statistical analysis

Response surface methodology has found be very useful and reliable method for optimizing the process parameters in many of the research experiments (Kaur *et al.*, 2018; Prema *et al.*, 2018; Balasubramanian *et al.*, 2020). It was used for designing of the experiment (Khuri and Cornell 1987). The software package Design Expert version 10 was used to generate the experimental designs, statistical analysis and regression models. A three-factor five-level Central Composite Rotatable Design (CCRD) with quadratic

model was employed to study the combined effect of three independent variables, viz., enzyme concentration, incubation temperature and incubation time on different response variables. The response function (Y) was related to the coded variables by a second order polynomial equation which can be given as under.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

The coefficients of 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). Analysis of variance (ANOVA) tables were generated and regression coefficients of individual linear, quadratic and interaction terms were determined (Khuri and Cornell 1987).

### Optimization and validation of process variables

The optimization of process variables was carried out using Design Expert software. Optimum values of the selected variables were analyzed by the response surface contour plots and also by solving the regression equation. The optimum condition as obtained through response surface analysis was verified by conducting the experiments in triplicate. The average experimental value of different response variables was used to check the validity and adequacy of the predicted models.

## RESULTS AND DISCUSSION

### Effect of enzymatic treatment on clarity of juice

The effect of enzyme concentration, temperature and time on clarity of prickly pear juice is presented in the Table 1. Fig 1a showed that the clarity of juice was increased with an increase in the enzyme concentration and incubation temperature up to 0.087% and 43°C, respectively. It was expected to be increased up to 50.94%T at this combination of enzyme concentration and temperature. The perusal of contour map (Fig 2a) indicated that the clarity of juice was increased with an increase in incubation temperature up to 47°C and incubation time up to its maximum level of 180 min. This interaction level of temperature and time was proposed to increase the juice clarity up to 55.04%T. The interaction of enzyme concentration and incubation time caused to increase the clarity up to 54.81 %T (Fig 3a). The enzyme concentration and incubation time at this juncture was determined as 0.062% and 180 min, respectively. The higher incubation time provided the sufficient time to pectinase for breakdown of pectin which was the main phenomenon responsible for increase in juice clarity after prolonged time (Karangwa *et al.*, 2010). The resultant model, giving the empirical relation between the clarity of juice and the test variables in coded units, was obtained as under.

$$\text{Clarity} = 49.51 + 0.285X_1 - 10968X_2 + 1.983X_3 - 0.787X_1^2 - 1.975X_2^2 + 0.641X_3^2 - 1.806X_1X_2 + 0.271X_1X_3 + 0.111X_2X_3 \quad (3)$$

### Effect of enzymatic treatment on color index of juice

The data as tabulated in Table 1 as well as the interaction

plots of color index of prickly pear juice (Fig 1b) showed the positive effect of enzyme concentration and incubation temperature up to 0.047% and 44°C, respectively. This interaction was expected to increase the color index of juice up to 0.612 abs. However, with further increase in enzyme concentration and incubation temperature, the color index was decreased. Similarly, the positive effect of interaction of incubation temperature and time on color index of prickly pear juice was observed up to 44°C and 126 min, respectively. It was likely to be increased up to 0.612 abs at this combination of temperature and time (Figure 2b). Further increase in incubation temperature and time reduced the color index of juice. The interaction of enzyme concentration and incubation time, was effective up to 0.042% and 131 min, respectively. This interaction was expected to improve the color index of juice up to 0.596 abs (Figure 3b). But, the color index was decreased with further rise in enzyme concentration and incubation time. The regression model as derived for the color index of juice is given as under.

$$\text{Color index} = 0.593 - 0.009X_1 - 0.034X_2 + 0.008X_3 - 0.016X_1^2 - 0.016X_2^2 + 0.017X_3^2 - 0.005X_1X_2 + 0.008X_1X_3 + 0.002X_2X_3 \quad (4)$$

#### Effect of enzymatic treatment on betalain content

The data showing the effect of enzyme concentration, temperature and time on betalain content of clarified prickly pear juice are tabularized in Table 1. The response surface

and contour plot for the betalain content of juice as a function of enzyme concentration and incubation temperature (Figure 1c) revealed that betalain content of juice was increased with an increase of enzyme concentration up to 0.05 % and incubation temperature up to 47°C, respectively. For this interaction, it was possible to increase the betalain content of juice up to 544.80 mg/L. The contour map for betalain content (Fig 2c) indicated that interaction of incubation temperature and time positively affected the betalain content till 47°C and 128 min, respectively. The betalain content of the juice was expected to be increased up to 544.90 mg/L of juice at this combination of temperature and time. The betalain content was found to be decreased with further increase in temperature and time. For the third interaction as shown Fig 3c, the betalain content was found to be increased up to 0.053% enzyme concentration and 147 min incubation time. This combination was expected to increase the betalain content up to 542.64 mg/L of juice. The following empirical model in coded form was derived for the betalain content.

$$\text{Betalain content} = 542.635 - 0.687X_1 - 8.237X_2 + 3.532X_3 - 5.356X_1^2 - 7.836X_2^2 + 2.957X_3^2 - 1.498X_1X_2 + 0.41X_1X_3 + 5.025X_2X_3 \quad (5)$$

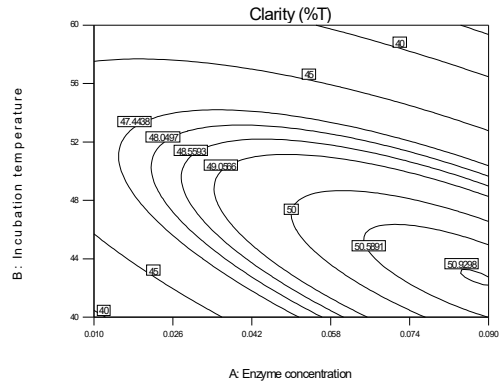
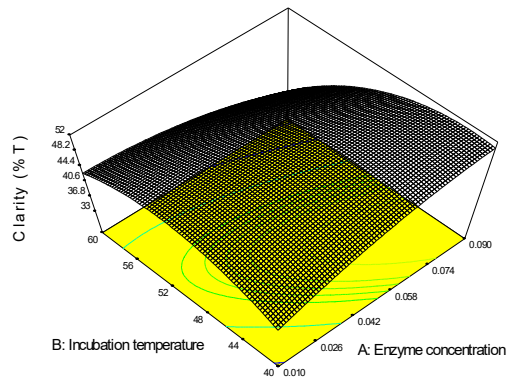
#### Effect of enzymatic treatment on ascorbic acid

Increase of enzyme concentration and incubation temperature, could play the beneficial role only up to 0.062%

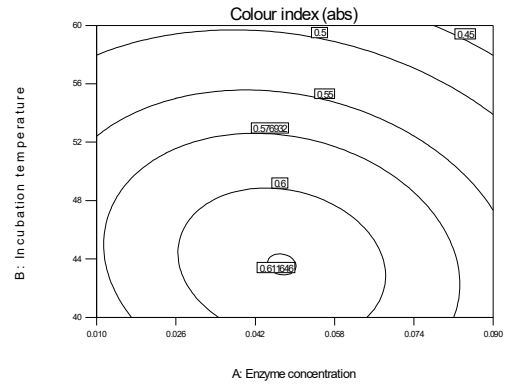
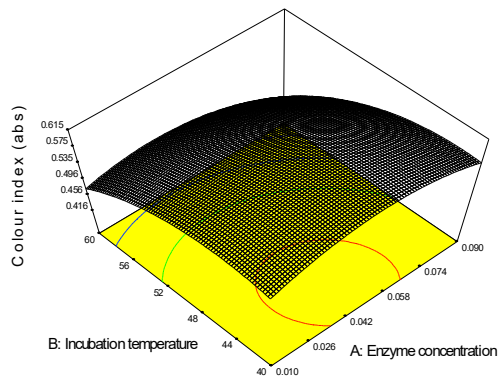
**Table 1:** Effect of enzyme concentration, incubation temperature and incubation time on response variables of clarified prickly pear juice

Treatment	Independent Variables			Responses.			
No.	Enzyme Conc. (%)	Temp. (°C)	Time (min)	Clarity (%T)	Color index (abs)	Betalain content (mg/L of juice)	Ascorbic acid (mg/100 g)
	$X_1(x_1)$	$X_2(x_2)$	$X_3(x_3)$	$Y_1$	$Y_2$	$Y_3$	$Y_4$
1	0.026(-1)	44(-1)	84(-1)	46.31	0.581	538.51	9.56
2	0.074(1)	44(-1)	84(-1)	50.02	0.580	537.21	9.28
3	0.026(-1)	56(1)	84(-1)	45.75	0.502	508.91	9.23
4	0.074(1)	56(1)	84(-1)	42.12	0.497	511.62	8.95
5	0.026(-1)	44(-1)	156(1)	49.14	0.601	536.56	8.99
6	0.074(1)	44(-1)	156(1)	53.82	0.585	534.92	9.32
7	0.026(-1)	56(1)	156(1)	48.91	0.544	525.08	8.66
8	0.074(1)	56(1)	156(1)	46.48	0.494	531.41	8.85
9	0.01(-1.682)	50(0)	120(0)	46.21	0.558	524.33	9.14
10	0.09(1.682)	50(0)	120(0)	47.14	0.531	526.28	9.10
11	0.05(0)	40(-1.682)	120(0)	46.54	0.591	530.87	9.28
12	0.05(0)	60(1.682)	120(0)	40.09	0.498	505.71	8.63
13	0.05(0)	50(0)	60(-1.682)	46.87	0.528	527.18	9.23
14	0.05(0)	50(0)	180(1.682)	54.56	0.555	537.00	8.71
15	0.05(0)	50(0)	120(0)	48.92	0.592	540.95	9.25
16	0.05(0)	50(0)	120(0)	49.27	0.588	544.00	9.27
17	0.05(0)	50(0)	120(0)	48.70	0.600	544.67	9.24
18	0.05(0)	50(0)	120(0)	50.49	0.582	540.06	9.16
19	0.05(0)	50(0)	120(0)	49.22	0.595	543.73	9.23
20	0.05(0)	50(0)	120(0)	50.67	0.603	543.15	9.21

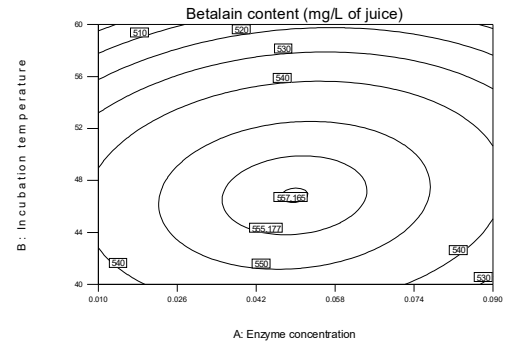
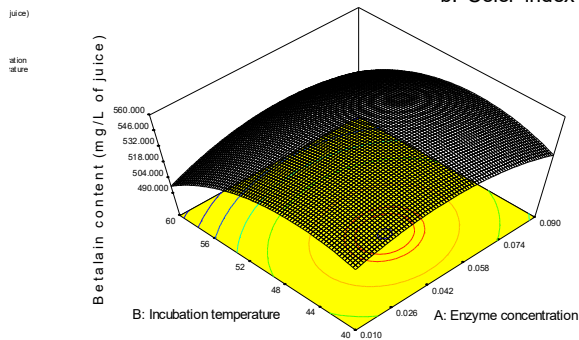
**Note:** value s in parenthesis are coded value.



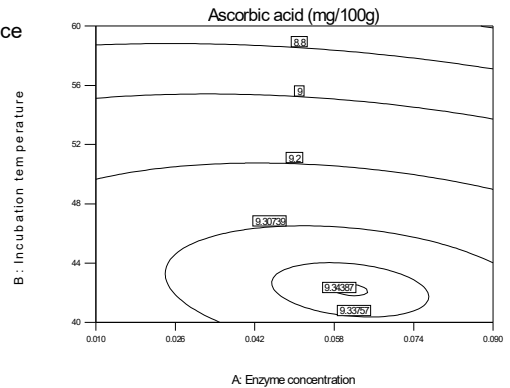
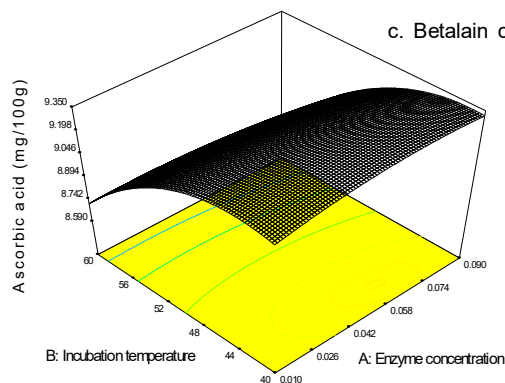
a. Clarity of juice



b. Color index of juice



c. Betalain content of juice



d. Ascorbic acid content of juice

**Fig 1:** Interaction effect of enzyme concentration and incubation temperature on clarity, color index, betalain content and ascorbic acid (Incubation time was at the centre point, i.e. 120 min).

and 42°C, respectively and expected to increase the ascorbic acid up to 9.34 mg/100 g (Fig 1d). Increase in ascorbic acid was observed with the increase of incubation temperature and time up to 43°C and 86 min, respectively (Fig 2d). This interaction predicted the rise in ascorbic acid up to 9.40 mg/100 g. The interaction effect as presented through contour graph (Fig 3d) revealed that there was decrease in ascorbic acid in the juice with an increase in both enzyme concentration and incubation time. Incubation time had shown its significant effect in decreasing ascorbic acid in comparison to enzyme concentration. Therefore, the lowest ascorbic acid (8.36 mg/100g) was observed at combination of 0.01% enzyme concentration and 180 min incubation time. While, the highest (9.63 mg/100g) was observed for the interaction of 0.01% enzyme concentration and 60 min incubation time. The model as derived and giving the empirical relation between ascorbic acid value and test variables in coded units, was obtained as under.

Ascorbic acid =

$$9.223 - 0.008X_1 - 0.187X_2 + 0.152X_3 - 0.016X_1^2 - 0.074X_2^2 + 0.069X_3^2 - 0.018X_1X_2 + 0.135X_1X_3 + 0.018X_2X_3 \quad (6)$$

#### Fitting the model

The different models for the different dependent variables, viz. clarity, color index, betalain content and ascorbic acid, were derived as represented by the Eq. 3, Eq. 4, Eq. 5 and Eq. 6, respectively. The values of different indicators for the

model fitting were obtained for these selected parameters as reported in the Table 2. The calculated F-value was significant at  $p < 0.001$  for all the selected responses. At the same time, all these parameters possessed non-significant lack of fit ( $p > 0.05$ ). These values indicated that the models as derived for all the selected parameters were fitted and reliable. The  $R^2$  and Adj- $R^2$  value for all the responses as determined through regression analysis were higher than the 0.8, which demonstrated the adequacy, good fit and high significance of the model. The Pred- $R^2$  value for all the factors was in reasonable agreement with the Adj- $R^2$ . The high Adeq Precision value ( $> 4$ ) again supported the significance of all the derived models. The small value of coefficient of variation for all the responses explained that the experimental results were precise and reliable (Table 2).

#### Optimization of process variables

Among the different independent variables, enzyme concentration has the greatest impact on the processing cost. To economize the process, minimum enzyme concentrations were used as criteria in numerical optimization and ideal operational conditions were derived. Accordingly, the goals that were set for variables and responses to obtain the optimum processing condition are illustrated in the Table 3. Under these constraints, the optimum treatment conditions were found to be, 0.036 % (w/w) enzyme concentration, 46°C incubation temperature and 112 min incubation time. The analysis showed that at

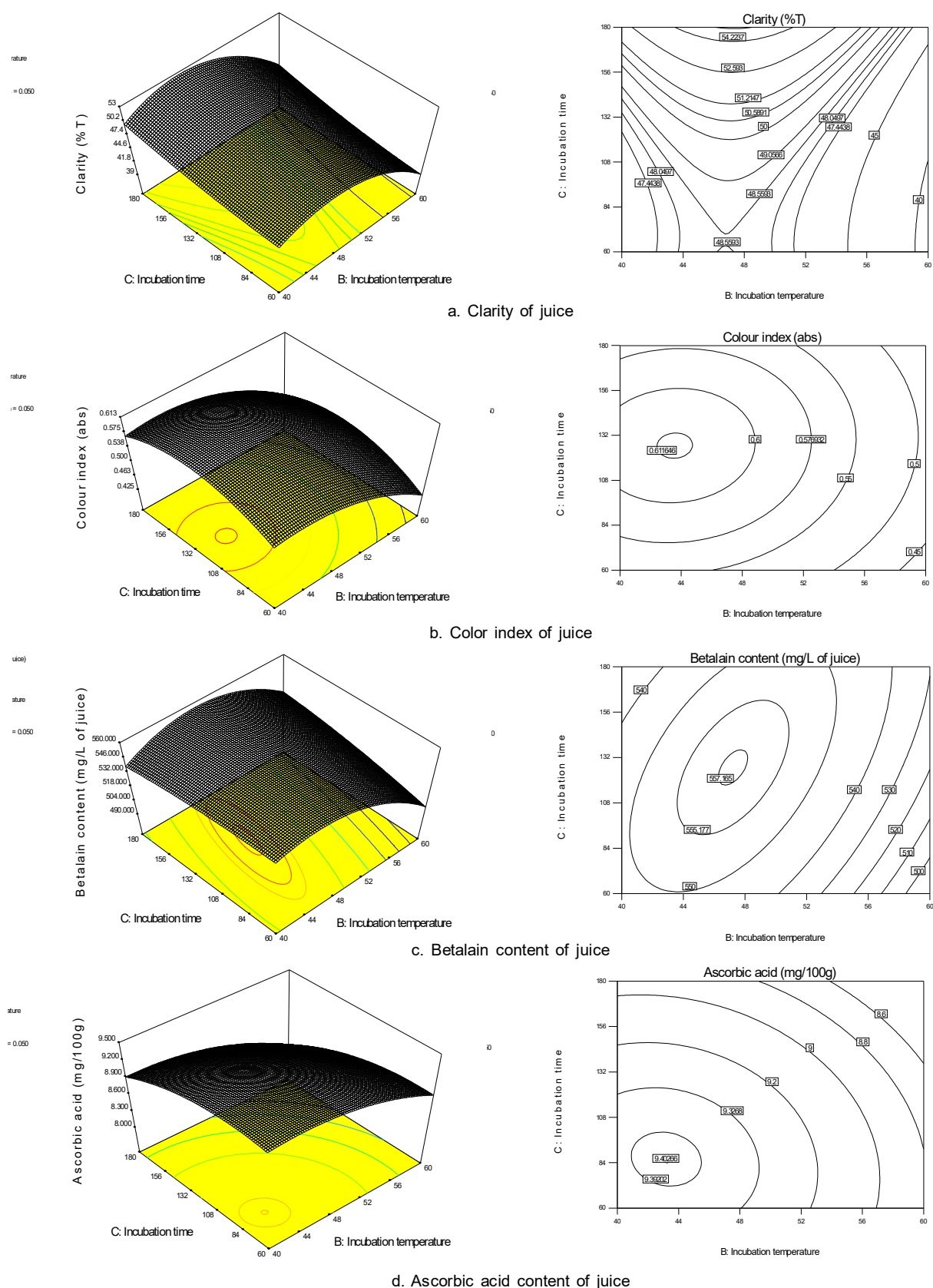
**Table 2:** ANOVA table for different quality characteristics of clarified prickly pear juice.

Source	Clarity (%T)	Color index (abs)	Betalain content (mg/L of juice)	Ascorbic acid (mg/100 g)
Model	49.51***	0.593**	542.63***	9.223***
Linear terms				
$b_1 (X_1)$	0.285	-0.009*	0.69	-0.008
$b_2 (X_2)$	-1.968***	-0.034***	-8.24***	-0.187***
$b_3 (X_3)$	1.983***	0.008*	3.53***	-0.152***
Interaction terms				
$b_{12} (X_1X_2)$	-1.806***	-0.005	1.50	-0.018
$b_{13} (X_1X_3)$	0.271	-0.008	0.41	0.135***
$b_{23} (X_2X_3)$	0.111	0.002	5.03***	-0.018
Quadratic terms				
$b_{11} (X_1^2)$	-0.787***	-0.016***	-5.36***	-0.016
$b_{22} (X_2^2)$	-1.975***	-0.016***	-7.84***	-0.074**
$b_{33} (X_3^2)$	0.641*	-0.017***	-2.96**	-0.069**
Indicators for model fitting				
$R^2$	0.963	0.965	0.971	0.961
Adj- $R^2$	0.929	0.934	0.945	0.925
Pred- $R^2$	0.815	0.793	0.821	0.740
Adeq. Precision	22.11	16.21	18.51	19.42
F-value	28.56	30.71	37.57	27.15
Lack of Fit	NS	NS	NS	NS
C.V., %	1.87	1.79	0.52	0.73

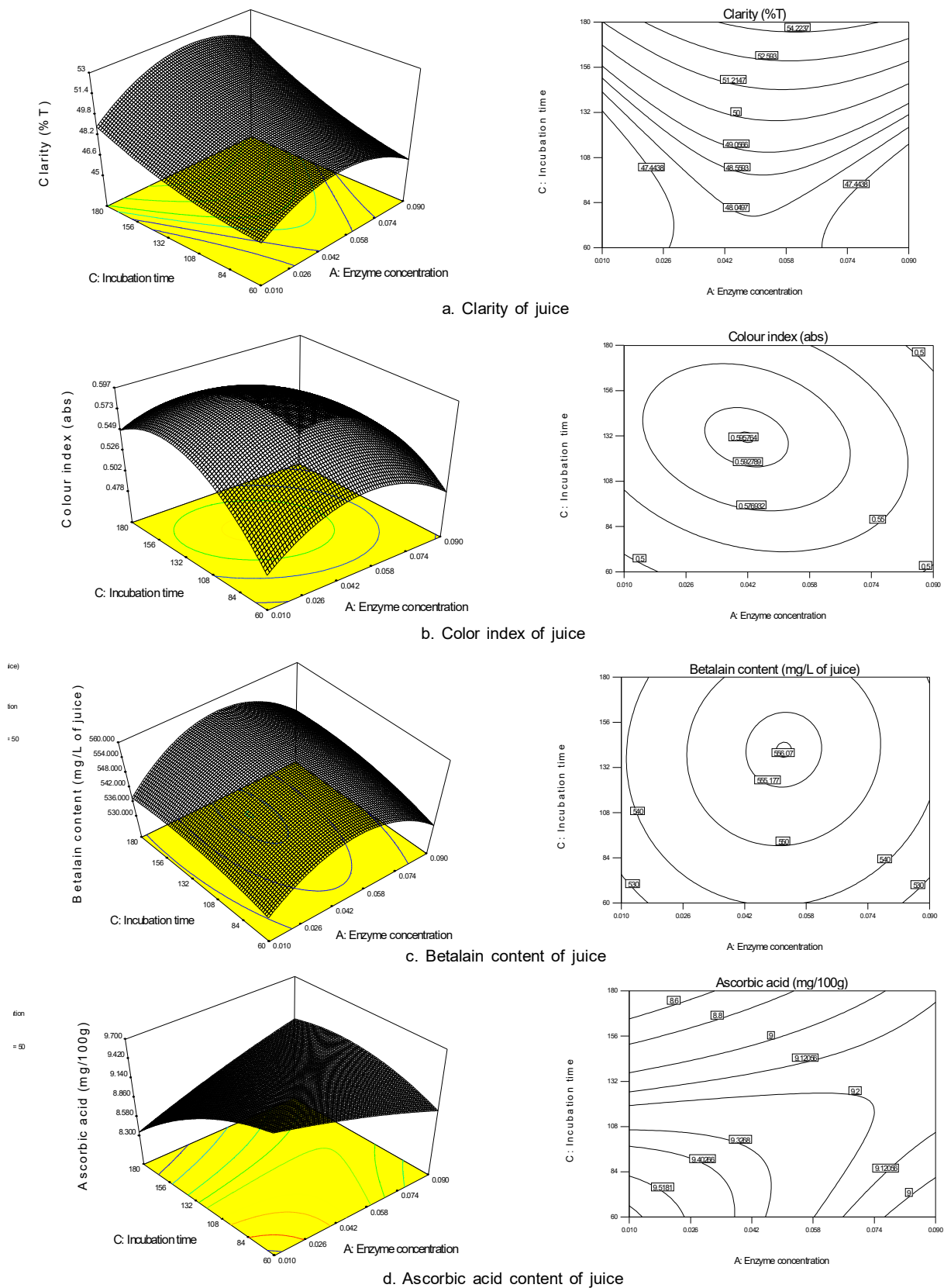
$X_1$  = Enzyme concentration,  $X_2$  = incubation temperature,  $X_3$  = Incubation time.

\*\*\*Significant at  $p < 0.001$ , \*\*Significant at  $p < 0.01$ , \*Significant at  $p < 0.05$ .





**Fig 2:** Interaction effect of incubation temperature and incubation time on clarity, color index, betalain content and ascorbic acid (Enzyme concentration was at the centre point, *i.e.* 0.05%).



**Fig 3:** Interaction effect of enzyme concentration and incubation time on clarity, color index, betalain content and ascorbic acid (Incubation temperature was at the centre point, i.e. 50°C).

**Table 3:** Numerical optimization of prickly pear juice clarification.

Variables				
Constraint	Goal	Optimum value		
Enzyme concentration (%)	Minimize	0.036		
Incubation temperature (°C)	In the range	46.4 (46)		
Incubation time (min)	In the range	112.4 (112)		
Responses				
Constraint	Goal	Predicted value	Experimental value	Deviation (%)
Clarity (%T)	Maximize	48.59	47.86	1.50
Color index (abs)	Maximize	0.603	0.598	0.83
Betalain content (mg/L of juice)	Maximize	542.93	536.54	1.18
Ascorbic acid (mg/100 g)	Maximize	9.346	9.14	2.20
Desirability	-	0.780	-	-

this combination of enzyme concentration, incubation temperature and incubation time, it would be possible to produce the juice with a clarity of 48.59% T, color index of 0.603 abs, betalain content of 542.93 mg/L of juice and ascorbic acid of 9.35 mg/100 g. Using these optimized conditions, the experiments were again conducted to find the variation in the different response variables. The results revealed that the experimental values of conducted experiments were very close to the predicted values (Table 3). This implied that there was a high degree of fit between the observed and predicted values from the regression models and each model was quite accurate in prediction. The closeness of the observed and predicted responses indicated the validity of developed model.

## CONCLUSION

The commercial pectinase enzyme presented an excellent result for improving the clarity of prickly pear juice with better retention of other quality parameters. The different process conditions for enzymatic treatment revealed that all these selected variables noticeably affected the clarity, color index, betalain content and ascorbic acid content of the prickly pear juice. The second order polynomial equations adequately described the relations between the processing and response variables. The response surface and contour plots assisted to study the interaction effect of process variables on response variables as well as to obtain the optimum levels for enzymatic pretreatments. The optimum condition for clarification of prickly pear juice could be recommended as: enzyme concentration of 0.036%, incubation temperature of 46 °C and incubation time of 112 min. The obtained result would be beneficial for juice industry to improve the clarity and color index along with preservation of betalain content and ascorbic acid of the prickly pear fruit juice.

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