Response Surface Modeling of Prickly Pear Juice Clarification

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Abstract. Raw juice of prickly pear is viscous and turbid in nature. The clarity of such juice can be improved through enzymatic clarification. An experiment was conducted to standardize the process parameters for clarification of prickly pear juice using pectinase enzyme. Experiment was designed according to response surface methodology (RSM) by keeping three selected independent variables at five different levels, i.e. enzyme concentrations (0.01, 0.026, 0.050, 0.074 and 0.09%), incubation temperatures (40, 44, 50, 56 and 60 °C), and incubation time (60, 84, 120, 156 and 180 min). Second order central composite rotatable design was employed to study the effect of enzymatic treatments on yield, viscosity, clarity, and turbidity of juice. Response surface modeling showed that the generated regression models were adequate to explain the data variation as well as the actual relationship between independent variables and responses. The coefficient of determination, R² values for all the selected parameters were greater than 0.9. Through response surface analysis, the optimum conditions for enzymatic clarification of prickly pear juice were established as 0.056% enzyme concentration, 47 °C incubation temperature, and 155 min incubation time. This interaction of process parameters was able to improve the yield by up to 88.83% and clarity up to 52.86% as well as decreasing the viscosity by up to 1.60 cP, and turbidity by up to 123.02 NTU.

Keywords: Prickly pear, response surface modelling, juice clarification, pectinase enzyme, optimization

1 Introduction

Prickly pear belongs to the Cactaceae family. It is a wild fruit which grows under arid and semiarid conditions [34], [12], [30]. The demand of nutraceuticals, natural ingredients and health-promoting foods is continuously increasing. The variety of functional properties retained by prickly pear fruit fits well in this trend [30]. Fruit juices are very important in improving the human health [35]. The fruit juices market has substantially grown mainly due to the current trend of involving naturally healthy foods in our daily diets [49]. Prickly pear juices are rich in amino acids contents, particularly proline and taurine, and minerals such as calcium and magnesium which are considered as valuable ingredient for sports and energy drinks [36],[33]. The dark red colour of prickly pear juice makes it a valuable source for enhancing colour in fruit juice blends [27]. Prickly pear fruit has therefore received renewed interest for the production of juice.

The raw fruit juices contain suspended solids like earth, skin, stem and cellular debris from the fruit which makes it very turbid and disliked by the consumers. But, clarity of juice is of utmost importance from the consumers point of view. The particles suspended in the prepared juice spoil the presentation as well as affect the flavour of juice. The presence of polysaccharides such as pectin and starch is responsible for turbidity in the juice. Such juices had more characteristics of a puree than of a beverage and drinkability of it is reduced [37]. The clarity of the juices can be improved by removing the large pieces of debris through centrifugation. Though, it has not been found effective in the juice clarification and most of the small particles always remain in suspension. These suspended particles have to be removed to get a clear juice. Filtration is the alternative to remove these suspension, but some soluble pectin still remains in the juice, making it too viscous and difficult to filter quickly [23]. The complete enzymatic breakdown of pectin is the efficient way to produce clear and stable juice [21]. The most accepted method for removing the unwanted pectin is treating the fruit juices with pectinase [4], [6]. The controlling factors affecting the enzymatic hydrolysis of pectic substances are type of enzyme,
enzyme concentration, hydrolysis time, incubation temperature and pH [21], [40]. These factors are to be optimized to improve the recovery along with quality of juice. In view to this, the study was conducted to develop the protocol for clarification of prickly pear juice. The response surface modelling was done to establish the relationship between independent and response parameters.

Response surface methodology (RSM) has been used extensively by the researchers for optimizing the process parameters in the fruit juice production [21], [40], [50], [52]. It reduces the number of experimental trials that need to evaluate multiple parameters and their interactions. In comparison to other approaches, it is less laborious and time-consuming. It has successfully demonstrated its usefulness in optimizing ingredients [8], [38], [48] and process variables [14], [24], [29], [39], [43], [47] or both [10], [52]. The aim of the study was to establish the optimum process conditions (enzyme concentration, incubation temperature and incubation time) for enzymatic clarification of prickly pear juice using response surface methodology and to study the effect of enzymatic treatments on yield, clarity, viscosity and turbidity of juice.

2 Material and Methods

2.1 Fruit

The fresh prickly pear fruit was collected from the plants grown at locally available farms and nearby areas of Junagadh (Gujarat, India). Fully ripe, bright red and purple colour fruits, without any visual defects, were selected for the experimental work.

2.2 Enzyme Source

Pectinase enzyme was used for the clarification of juice. It was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai (Maharashtra, India) and was stored under refrigerated condition at 4 °C. The activity of pectinase enzyme is 8000-12000 U/g with an optimum pH range of 5.0-5.5.

2.3 Raw Material Preparation

The fruits selected for the experiment were made free from the thorn by burning its thorn on flame and brushing them carefully on the abrasive surface without any damage to fruit. The dust and burn thorn particles on the fruit surface were then removed by washing it in the cool tap water. The damaged, defective, and over ripened fruits were not taken for the experimental work.

2.4 Extraction of Pulp

The cleaned prickly pear fruits were manually cut longitudinally into two halves to facilitate removal of seed and sub pulping. The longitudinally cut pulp was then scooped out with a sanitized spoon. Scooped pulp consisting of both, pulp and seeds, was put into a mixture grinder at low speed for 10-15 seconds just to facilitate the separation of seed from pulp. Enough care was taken during the grinding process to avoid the breakage of seeds. Whole mixture was then transferred to a domestic sieve having sieve size of 8 mesh for the separation of seeds from the pulp. The pure pulp without any seeds was finally used in the clarification process.

2.5 Enzymatic Clarification

The pure pulp of prickly pear fruit was very turbid due to presence of suspended pulp tissues as well as polysaccharides such as pectin and sugar. 100 g pulp was subjected to different enzymatic treatments under different conditions as shown in Table 1. The independent variables were enzyme concentration, X1 (0.01-0.09%), incubation temperature, X2 (40-60 °C) and incubation time, X3 (60-180 min). The flow chart in Fig. 1, illustrates the method used for enzymatic clarification of prickly pear juice.
Table 1. Experimental design indicating coded and actual values of independent variables for enzymatic clarification of prickly pear juice.

| Treatment No. | Coded values | Actual values | Enzyme concentration (%w/w) | Temperature (°C) | Time (min) |
|---------------|--------------|---------------|----------------------------|-----------------|------------|
|               | X₁ | X₂ | X₃ |                             |                |            |
| 1             | -1 | -1 | -1 | 0.026                        | 44             | 84         |
| 2             | 1  | -1 | -1 | 0.074                        | 44             | 84         |
| 3             | -1 | 1  | -1 | 0.026                        | 56             | 84         |
| 4             | 1  | 1  | -1 | 0.074                        | 56             | 84         |
| 5             | -1 | -1 | 1  | 0.026                        | 44             | 156        |
| 6             | 1  | -1 | 1  | 0.074                        | 44             | 156        |
| 7             | -1 | 1  | 1  | 0.026                        | 56             | 156        |
| 8             | 1  | 1  | 1  | 0.074                        | 56             | 156        |
| 9             | -1.682 | 0 | 0  | 0.010                        | 50             | 120        |
| 10            | 1.682 | 0 | 0  | 0.000                        | 50             | 120        |
| 11            | 0   | -1.682 | 0 | 0.050                        | 40             | 120        |
| 12            | 0   | 1.682 | 0 | 0.050                        | 60             | 120        |
| 13            | 0   | 0   | -1.682 | 0.050                        | 50             | 60         |
| 14            | 0   | 0   | 1.682 | 0.050                        | 50             | 180        |
| 15            | 0   | 0   | 0  | 0.050                        | 50             | 120        |
| 16            | 0   | 0   | 0  | 0.050                        | 50             | 120        |
| 17            | 0   | 0   | 0  | 0.050                        | 50             | 120        |
| 18            | 0   | 0   | 0  | 0.050                        | 50             | 120        |
| 19            | 0   | 0   | 0  | 0.050                        | 50             | 120        |
| 20            | 0   | 0   | 0  | 0.050                        | 50             | 120        |

2.6 Juice Characteristics

2.6.1 Juice Yield
The juice yield was calculated by taking the ratio of weight of clear juice and pulp weight. It was recorded in percent of juice.

\[
\text{Percent juice yield} = \left( \frac{\text{Weight of clear juice}}{\text{Weight of pulp}} \right) \times 100
\]

2.6.2 Viscosity
The viscosity measurement of clarified prickly pear juice samples was made using Brookfield Rheometer (DV III Ultra, Brookfield Engineering Laboratory, Inc., Middleboro, USA). Viscosity reading was taken at room temperature (30±1 °C) by rotating spindle LV-2 at 100 rpm. It was recorded in centipoise (cP).

2.6.3 Clarity
The clarity of juice was determined by using UV-VIS spectrophotometer (UV 5704SS, Electronics Corporation of India Pvt. Ltd., Hyderabad, India). The spectrophotometer was set at a wavelength of 660 nm and percent transmittance (%T) was recorded by passing a light through the juice sample [21],[22]. Distilled water was used as a reference.
2.6.4 Turbidity

Turbidity of juice was measured using a portable turbidity meter (T-100, OAKTON Instruments, Vernon Hills, IL 60061, USA) at room temperature [40]. The calibration of the instrument was done using calibration standards before it is to be used for measurement. Juice sample was thoroughly mixed to get the suspended solids dispersed uniformly. It was then allowed to rest until all visible air bubbles disappeared. The sample was then poured into the cleaned sample vial up to the mark on the vial, capped and inverted twice to ensure even mixing. The vial containing the sample was wiped from outside, placed in the sample chamber and covered with vial cover. The measurement was then taken and expressed in Nephelometric Turbidity Units (NTU).

2.6.5 Experimental Design and Statistical Analysis

The experiment was designed adopting response surface methodology (RSM) [28],[18],[26]. The software package Design Expert version 8.0.0.6 (Trial version; STAT-EASE Inc., Minneapolis, MN, USA) 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 (X1), incubation temperature (X2) and incubation time (X3) on different response variables. The pH of the pulp was kept at its natural value, i.e. 5.05, and was not included in the RSM experimental design as this pH range is optimal for the exogenous pectinases [20]. Moreover, pH adjustment to alter clarification is not
practiced commercially as described by [32]. Therefore, it was excluded from the RSM experimental design. Total 20 different combinations (Table 1) including six replicates of the centre point were carried out in random order according to CCRD configuration for the three chosen variables. The response function (Y) was related to the coded variables by a second order polynomial equation given as below.

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_{11} + b_{22}X_{22} + b_{33}X_{33} + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3, \]  

(1)

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 the effect and regression coefficients of individual linear, quadratic and interaction terms were determined [18]. The significances of all terms in the polynomial were assessed statistically by computing the F-value at a probability (p) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculation and to generate contour maps from the regression models. The three-dimensional (3D) response surface plots were generated by keeping one variable constant at the centre point and varying the other two variables within the experimental range.

2.6.6 Optimization and Validation of Model

The Design Expert version 8.0.0.6 software was used for the optimization of process variables. The optimum values of the selected variables were analyzed by the response surface contour plots and also by solving the regression equation. The optimum conditions obtained through response surface analysis were verified by conducting the experiments in triplicate. The average experimental value of different response variables were used to check the validity and adequacy of the predicted models.

3 Results and Discussion

3.1 Statistical and Model Analysis

The experimental values for yield, viscosity, clarity, and turbidity of prickly pear juice under different treatment conditions are reported in Table 2. The result of the regression analysis and analysis of variance (ANOVA) for all the models is reported in Table 3. The significant F-value and non-significant lack of fit indicates the fitness and reliability of the model for a given response. However, the adequacy of the model needed to be further checked by the coefficient of regression (R^2) [11]. The closer the value of R^2 to unity, the better the empirical model fits the actual data. The value of R^2 greater than 0.8 implies that the model indicates a good fit [15]. Nevertheless, some researchers suggested that a large value of R^2 does not always imply that the regression model is a good one. Increasing R^2 can be obtained by adding a variable to the model. Thus, it is preferred to use an adjusted R^2 to evaluate the model adequacy and it should be more than 0.8 [19]. Moreover, other parameters, namely predicted R^2 which should be closer to value 1 and adequate precision which should be greater than 4 are supportive of the significance of the model [1]. The small values of C.V. give better reproducibility indicating the better precision and reliability of experiment. It is desirable to have a C.V. of less than 10%.

| Sr. No. | Independent Variables | Responses |
|---------|-----------------------|-----------|
|         | Enzyme Conc. (%)      | Temp. (°C) | Time (min) | Juice yield (%) | Viscosity (cP) | Clarity (%T) | Turbidity (NTU) |
| 1       | 0.026(-1)             | 44(-1)     | 84(-1)     | 86.67          | 1.68           | 46.31        | 153            |
| 2       | 0.074(1)              | 44(-1)     | 84(-1)     | 87.93          | 1.65           | 50.02        | 136            |
| 3       | 0.026(-1)             | 56(1)      | 84(-1)     | 87.93          | 1.66           | 45.75        | 177            |
| 4       | 0.074(1)              | 56(1)      | 84(-1)     | 87.26          | 1.67           | 42.12        | 169            |
| 5       | 0.026(-1)             | 44(-1)     | 156(1)     | 87.21          | 1.62           | 48.91        | 139            |
| 6       | 0.074(1)              | 44(-1)     | 156(1)     | 89.01          | 1.59           | 53.82        | 120            |
| 7       | 0.026(-1)             | 56(1)      | 156(1)     | 88.27          | 1.63           | 46.48        | 142            |
| 8       | 0.074(1)              | 56(1)      | 156(1)     | 88.27          | 1.63           | 46.48        | 142            |
Model analysis was done for checking its validity. Looking to the values of various statistical indicators, such as F-value, coefficient of determination (R²) and coefficient of variation (C.V.) as given in Table 3, all the models were found statistically adequate. The significant F-value (p<0.001) and non-significant lack of fit (p<0.05) for yield, viscosity, clarity and turbidity of prickly pear juice concluded that all the models were fitted. The higher R² and Adj-R² values (greater than 0.8) for all the selected responses indicated the adequacy, good fit and high significance of the model. The analysis of variance (ANOVA) of four response variables, i.e., yield, viscosity, clarity and turbidity showed that experimental data had correlation coefficients (R²) of 0.9740, 0.9708, 0.9626 and 0.9755, respectively with the calculated model. That means the calculated model was able to explain 97.40, 97.08, 96.26 and 97.55% of the results in case of yield, viscosity, clarity and turbidity, respectively. Pred-R² value was also in reasonable agreement with the Adj-R² for all the parameters. The Adeq Precision value greater than 4 further highlighted the significance of the model for all the dependent variables. The small value of C.V. (<10%) for all the variables explained that the experimental results were precise and reliable. Neglecting the non-significant parameters, the final predictive equations obtained were given as below:

\[
\text{Juice yield} = 88.323 + 0.592X_1 - 0.297X_2 + 0.468X_3 - 0.379X_1^2 - 0.278X_2^2 - 0.379X_1X_2
\]  
\[
\text{Viscosity} = 1.612 - 0.007X_1 + 0.004X_2 - 0.023X_3 + 0.968X_1^2 + 0.009X_2^2 + 0.013X_3^2 + 0.013X_1X_2
\]  
\[
\text{Clarity} = 49.51 - 1.968X_2 + 1.983X_3 - 0.787X_1^2 - 1.975X_2^2 + 0.641X_3^2 - 1.806X_1X_2
\]  
\[
\text{Turbidity} = 138.688 - 3.651X_1 + 10.308X_2 - 13.203X_3 + 5.104X_2^2 + 2.25X_1X_2 + 2.75X_1X_3 - 2.75X_2X_3
\]

where, X1, X2 and X3 are the coded factors of enzyme concentration, incubation temperature and incubation time, respectively.

**Table 3.** Regression coefficients, R² and p values for different response variables for enzymatic clarification of prickly pear juice.

| Source | Juice yield (%) | Viscosity (cP) | Clarity (ºT) | Turbidity (NTU) |
|--------|----------------|----------------|--------------|----------------|
| b0     | 88.323***      | 1.612***       | 49.51***     | 138.688***     |
| Linear terms | b1 (X1) | 0.592*** | -0.007*** | 0.285 | -3.651*** |
| | b2 (X2) | -0.297*** | 0.004*** | -1.968*** | 10.308*** |
| | b3 (X3) | 0.468*** | -0.023*** | 1.983*** | -13.203*** |
| Interaction terms | b12 (X1X2) | -0.032 | 0.013*** | -1.806*** | 2.25 |
| | b13 (X1X3) | -0.017 | -0.003 | 0.271 | 2.75 |
| | b23 (X2X3) | -0.025 | 0.002 | 0.111 | -2.75 |
| Quadratic terms | | | | |

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The following equation was obtained by the regression analysis

\[
\begin{align*}
\text{Juice yield (Y)} &= b_0 + b_1 \times X_1 + b_2 \times X_2 + b_3 \times X_3 + b_4 \times X_1^2 + b_5 \times X_2^2 + b_6 \times X_3^2 \\
&= 3.21 - 0.379 \times X_1 - 0.278 \times X_2 + 0.186 \times X_3 - 0.787 \times X_1^2 - 1.975 \times X_2^2 + 0.641 \times X_3^2
\end{align*}
\]

The indicators for the model fitting are shown in Table 2.

| Indicator                  | Value  |
|----------------------------|--------|
| R^2                        | 0.974  |
| Adj-R^2                    | 0.9505 |
| Pred-R^2                   | 0.8627 |
| Adeq. Precision            | 21.299 |
| F-value                    | 41.55  |
| Lack of Fit                | NS     |
| C.V., %                    | 0.21   |

\(X_1\) = 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

3.2 Effect of Enzyme Concentration, Temperature and Time

The effect of enzyme concentration, temperature, and time on the dependent variables is explained by the coefficient of the second order polynomials. The response surface curves as well as contour plots for different response variables are shown in Figure 2 to Figure 5.

3.2.1 Juice Yield

For the juice yield, the linear effect of enzyme concentration and incubation time was positive but the linear effect of incubation temperature was negative, which all were significant at p<0.001. Whilst, the quadratic effect enzyme concentration and incubation temperature, was significantly negative at p<0.001 and it was positively significant at p<0.01 for incubation time. However, all the interaction effects were found non-significant for the juice yield.

The interactive effect of enzyme concentration and temperature on the juice yield at constant time is presented in Fig. 2. Contour plot indicated that the juice yield was increased with an increase in enzyme concentration up to 0.069% and temperature up to 47 °C. At this combination the juice yield was expected to be improved up to 88.65%. Further rise in temperature decreased the yield. Slight decrease in yield was observed with an increase in enzyme concentration beyond 0.069%. The decrease in juice yield beyond 47 °C might be due to the denaturation of protein which led to decrease in enzyme activity. The increase in juice yield with increasing pectinase enzyme concentration was also supported by [31] who reported that pectinases degraded the pectic substances leading to increase in juice yield. [41] also found the increase in plum juice yield from 48% to 77.5% with an increase in enzyme concentration.

![Figure 2](image_url)

**Figure 2.** Three dimensional plot (a) and contour plot (b) for the effect of enzyme concentration and incubation temperature at constant time of 120 min on juice yield.

3.2.2 Viscosity
Fruit pulps generally present non-newtonian rheological behavior [45]. Researchers [3],[46] found that the use of enzymes leads to the drop of fruit juice viscosity, as well as improving pressability of the pulp, disintegrating the jelly structure and making it easier to obtain the fruit juices. The linear effect of viscosity was significantly affected by enzyme concentration and time at p<0.01 and p<0.001, respectively followed by temperature (p<0.05). The quadratic effects of all the independent variables were significantly positive at p<0.001. The interaction of enzyme concentration and temperature showed positive and significant effect at p<0.001 which indicated that the enzyme concentration was dependent on incubation temperature.

The response surface curve and contour plot showing the variation in the viscosity of juice as a function of enzyme concentration and temperature is presented in Fig. 3. The figure reflected the decrease in viscosity as the enzyme concentration increased up to 0.072% and temperature up to 45 °C. The viscosity at this combination was proposed to be decreased up to 1.61 cP. With further increase in enzyme viscosity, the viscosity of juice increased slightly whereas it increased linearly with an increase in temperature up to its maximum level. [16] reported the increase in viscosity of the blended carrot-orange juice with increase in temperature beyond 45 °C. [21] observed that the viscosity of juice decreased with increase in enzyme concentration from 0.01% to its maximum value (0.1%).

![Figure 3](image-url)

**Figure 3.** Three dimensional plot (a) and contour plot (b) for the effect of enzyme concentration and incubation temperature at constant time of 120 min on viscosity of juice.

### 3.2.3 Clarity

Clarity is one of the essential requirement for the clarified juice [46]. Clarified juice is a natural juice that is pulpless and do not have cloudy appearance [22]. Upon enzyme treatment, pectolytic enzymes breakdown the pectin molecules, which facilitate the formation of pectin-protein flocs, leaving a clear supernatant and significantly removing the colloidal aspect of the juices [2],[51]. The linear effects of time and temperature were significant (p<0.001) on clarity of juice. The quadratic effects of enzyme concentration, temperature and time were significant at p<0.001 or p<0.01. Only, the interaction effect between enzyme concentration and temperature was significant (p<0.001) which indicated the dependency of enzyme concentration on incubation temperature.

The interaction effect of temperature and time on clarity of juice as shown in Fig. 4, reveals 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.
The temperature increases the rate of enzymatic reactions, hence the rate of clarification, as long as the temperature is below denaturation temperature for the enzyme [21]. The clarity of juice was expected to be increased up to 59.94% at this combination of enzyme concentration and temperature. Upon further increase in enzyme concentration, the clarity of juice remained unaffected whereas it decreased considerably with an increase in temperature up to its maximum level [42] also found the analogous result on the clarity of juice from bael fruit. The degradation of polysaccharides like pectin led to a reduction in water holding capacity and consequently, free water was released to the system which increased the clarity of juice [6]. The other reason for the increase in clarity of juice was the increase in enzyme concentration. It may increase the rate of clarification by exposing part of the positively charged protein beneath. Thus, electrostatic repulsion between cloud particles was reduced which caused these particles to aggregate into larger particles and eventually settled out [40].

3.2.4 Turbidity

In clarified fruit juices, a juice that has an unstable cloud or whose turbidity is not desirable is considered “muddy” and is unacceptable to be marketed as clear juices [7]. Turbidity directly indicates unsettle matter or impurities in water suspension, such as colloidal polysaccharide particles in fresh juices [9]. For orange and tomato juices, this property is a positive sensorial aspect, whereas for pear, guava, apple and carambola juices it is a negative one [22],[5],[13],[17],[25],[40]. Table 3 reveals that enzyme concentration and time showed negative linear effect on turbidity at p<0.01 and p<0.001, respectively. The incubation temperature showed significantly positive effect on turbidity at p<0.001. The quadratic effect of incubation temperature was only significant at p<0.001. The interaction effects of time with enzyme concentration and temperature were significant at p<0.05. This means that the action of enzyme was dependent on the incubation temperature during enzymatic clarification of prickly pear juice.

In general, enzyme concentration was the main factor influencing the clarification of prickly pear juice. However, the temperature was found to be the equal important parameter, since the model showed a significant effect on linear, quadric and interactive regressions for turbidity. Fig. 5 shows three-dimensional and contour plot for the turbidity as a function of enzyme concentration and incubation temperature. The figure indicated that the turbidity of juice was decreased with an increase in the enzyme concentration up to its maximum level (0.09%) and incubation temperature up to 42 °C, respectively. At this combination, the turbidity of juice was believed to be reduced up to 126.27 NTU. The further increase in temperature showed an increase in turbidity. [9] stated that the pectin is the main cause of turbidity in the fruit juices. As the clarification process took place, the amount of pectin in the juices decreased, therefore reducing the turbidity of the juices [2]. In fact, long exposition to high enzyme concentrations were likely to breakdown pectic substances exposing positive nucleus sites to surrounding negative charges, settling out the so formed large protein-pectin particles [17].

Figure 4. Three dimensional plot (a) and contour plot (b) for the effect of enzyme concentration and incubation temperature at constant time of 120 min on clarity of juice.
3.3 Optimization and Validation of Process Variables

The process economy is a decisive factor needed to be taken into consideration in order to achieve a reasonable operational condition [44]. Among the different independent variables, enzyme concentration is the limiting factor affecting the cost of processing. To make the process economical with an ideal processing condition, optimization was carried out by keeping the criteria for enzyme concentrations at lower levels. As a consequence, the main criteria chosen for process optimization were: (a) enzyme concentration : minimum, (b) yield : maximum, (c) viscosity : minimum, (d) clarity : maximum, (e) turbidity : minimum as illustrated in Table 4.

Table 4. Optimization of process variables with respect to juice yield, viscosity, clarity and turbidity.

| Variables          | Constraint  | Goal           | Importance | Predicted value | Experimental value | Deviation (%) |
|--------------------|-------------|----------------|------------|-----------------|--------------------|---------------|
| Enzyme concentration (%) | Minimize     | 3              | 0.056      |                 |                    |               |
| Incubation temperature (°C) | In the range | 3              | 47         |                 |                    |               |
| Incubation time (min) | In the range | 3              | 155        |                 |                    |               |
| Juice yield (%)     | Maximize     | 3              | 88.83      | 87.16           | 1.88               |               |
| Viscosity (cP)      | Minimize     | 3              | 1.60       | 1.62            | 1.25               |               |
| Clarity (%T)        | Maximize     | 3              | 52.87      | 51.14           | 2.27               |               |
| Turbidity (NTU)     | Minimize     | 3              | 123.00     | 119.65          | 2.72               |               |
| Desirability        | --           | --             | 0.797      | --              | --                 |               |

Upon these inputs, the optimum treatment conditions were found to be, 0.056% enzyme concentration, 47 °C incubation temperature, and 155 min incubation time. The predictive study indicated that at this combination of enzyme concentration, incubation temperature and incubation time, it would be possible to produce juice with a yield of 88.83%, viscosity of 1.60 cP, clarity of 52.87%T and turbidity of 123.00 NTU. For these optimized conditions, the experiments were repeated to check the deviation in the predicted and experimental values of different response variables. Negligible and acceptable deviation was observed between experimental and predicted values. The experimental values were fairly close to the
predicted values (Table 4). 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 least variation in the actual and predicted responses justified that the model as developed from the experiment is valid.

4 Conclusions

The process variables (enzyme concentration, incubation temperature and incubation time) were optimized for enzymatic clarification of prickly pear juice. Regression models were generated to establish the correlation between independent variables and response parameters. The different combinations of controlled factors showed their noticeable effect on the juice yield, viscosity, clarity, and turbidity of the prickly pear juice. The yield and clarity were improved while the viscosity and turbidity were decreased upon enzymatic treatment. The optimum combination of processing variables can be obtained graphically in the form of three dimensional and contour plots through response surface analysis. It may be helpful to derive the suitable pretreatment levels for efficient clarification of prickly pear juice. The enzyme concentration of 0.056%, incubation temperature of 47 °C and incubation time of 155 min are suggested to obtain the better quality of prickly pear juice.

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