Optimization of enzymatic hydrolysis of pigeon pea for cooking quality of dhal

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Abstract: Cooking quality of dehusked splits is influenced by the dehulling method, in particular, by the pre-milling treatments. The effects of four enzymatic hydrolysis parameters, i.e., enzyme concentration (20–60 mg/100 g dry matter), incubation time (3-15 h), incubation temperature (40-60°C) and tempering water pH (4.0-6.0) on cooking time of pigeon pea dhal were optimized using response surface methodology. Three kinds of enzymes, i.e., xylanase, pectinase, and cellulase were used in combination for enzymatic pre-treatment. A quadratic model satisfactorily described the dehulling efficiency with high value for the coefficient of determination R² (0.9062). It predicted a minimum cooking time of 21.91 min at enzyme concentration of 37.8 mg/100 g dry matter, incubation time 8.69 min, incubation temperature 48.5°C and pH 5.49 of tempering water. Cooking time at optimum condition was observed to be 21.50 min and the predicted values of cooking time showed 2.19% deviation from the experimental values. Results of the study revealed that cooking time of enzyme treated dhal could be decreased by 19.77% compared to the oil treated dhal.

Keywords: pigeon pea, enzymatic hydrolysis, cooking quality, enzymatic pre-treatment, dehulling, response surface methodology

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1 Introduction

Pigeon pea ( Cajanus cajan L.) is one of the important pulse crops of India contributing 20.87% to the total production of all pulses[¹]. It is mostly consumed after dehulling in the form of dhal (decroricated split cotyledon). At present, consumers demand dhal to be cooked well in minimum possible time and have a good taste and flavour. The cooking time, widely accepted as an indicator of cooking quality, is mainly affected by starch, compactness of seed coat, endosperm and internal structure of grain[²]. Cooking improves the bioavailability of nutrients and also destroys some of the anti nutritional factors. During pre-milling treatment, enzymatic action leads to the structural changes and therefore cooking time may be affected. Long cooking time results in a decrease in protein quality and a loss of vitamins and minerals. The price for the dhal is fixed on the basis of its nutritional and cooking quality. The suggested method will increase the profit of the pulse milling industries/processors. Hence enzyme treated pigeon pea dhal requires a detailed study which could reduce the cooking time of dhal.
The cooking quality of enzyme treated pigeon pea dhal on different aspects were reported by Saxena and Srivastava\textsuperscript{3}, Singh\textsuperscript{4}, Singh et al\textsuperscript{5}, Deshpande et al\textsuperscript{6} and Srerama et al\textsuperscript{7}. The mechanism of enzymatic activity is controlled by four interacting parameters, i.e., enzyme concentration, incubation time, incubation temperature and pH of tempering water\textsuperscript{8}. Optimum levels of these parameters are necessary to get maximum recovery and better quality of dhal. Information on the effects of above parameters on cooking quality appears to be lacking. No systematically designed research approach for optimization of hydrolysis of process parameters for cooking quality of dhal has been attempted. Hence, it was considered necessary to optimize the pre-treatment parameters of enzymatic hydrolysis on different aspect, i.e., pH, enzyme concentration, incubation time and incubation temperature of pigeon pea for better cooking quality of dhal.

2 Material and methods

2.1 Selection of variety

Amongst different varieties of pigeon pea being cultivated in Gujarat, the BDN 2 variety is widely grown by the farmers throughout the state. Moreover, BDN 2 variety is milled in the pulse mills of Gujarat on large scale for getting pigeon pea dhal. In view of this, BDN 2 variety of pigeon pea was selected for the present investigation. The pigeon pea grain used for the study was procured from Sagdividi Farm of Junagadh Agricultural University, Junagadh, Gujarat, India.

2.2 Dehusking machine

The laboratory scale dehusking machine based on CIAE dhal mill design and fabricated by Bharodia\textsuperscript{9} with overall dimensions of 600 mm × 620 mm × 935 mm, capacity of 85 kg/h and power unit of 1 hp electric motor was used for all the milling studies. The optimum operating speed and feed rate of the dehusking machine were 1 420 rpm and 64 kg/h, respectively.

2.3 Selection of enzymes

The selection of enzymes was made on the basis of the chemical composition and binding substances present between husk and cotyledon of pigeon pea grain. The xylanase enzyme is widely used as bio-bleaching agent for lignin isolation\textsuperscript{10}. Cellulase and pectinase break down cellulose to beta-glucose and pectin to pectic acid, respectively. Thus, the xylanase, cellulase and pectinase are the key enzymes which rupture the binding materials leading to increase the dehulling efficiency. The xylanase was procured from Advanced Enzyme Technologies Ltd., Thane (Maharashtra) while cellulase and pectinase were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai (Maharashtra).

2.4 Standardization of ratio of enzymes

Preliminary trials were undertaken to arrive at standard proportions of enzymes, i.e., xylanase : pectinase : cellulase for maximizing the husk removal. Initially, the proportion was selected arbitrarily. The effect of selected enzyme combination on husk removal of pigeon pea grain was evaluated keeping the enzyme concentration, incubation time, incubation temperature and pH constant based on the technical specifications of the products provided by manufacturer (Table 1).

<table>
<thead>
<tr>
<th>Specification</th>
<th>Xylanase</th>
<th>Pectinase</th>
<th>Cellulase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Off white</td>
<td>Off white</td>
<td>Light brown</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
<td>Soluble in water</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Storage condition, °C</td>
<td>2-8</td>
<td>2-8</td>
<td>2-8</td>
</tr>
<tr>
<td>Optimum temperature range, °C</td>
<td>30-60</td>
<td>45-50</td>
<td>40-50</td>
</tr>
<tr>
<td>Optimum pH range</td>
<td>4.5-5.5</td>
<td>5.0-5.5</td>
<td>4.0-5.0</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>12.5 u/mg</td>
<td>---</td>
<td>≥ 10 u/mg</td>
</tr>
</tbody>
</table>

Results showed that the enzyme proportion of xylanase : pectinase : cellulase as 2:1:1 (50%:25%:25%) gave the maximum husk removal and thereby the maximum hulling efficiency. Following equations were used to calculate husk removal and hulling efficiency\textsuperscript{11}.

\[
\text{Husk removed (HR), } \% = \frac{HRd}{Ht} \times 100 \quad (1)
\]

\[
\text{Coefficient of hulling (Ch) } = 1 - \frac{Wuh}{Wth} \quad (2)
\]

\[
\text{Coefficient of wholeness of kernel (Cwk) } = \frac{Wfp}{Wfp + Wbr + Wpo} \quad (3)
\]

\[
\text{Hulling efficiency (HE) } = \text{Ch} \times \text{Cwk} \times 100 \quad (4)
\]

where, HRd = Husk removed during dehusking, g; Ht = Total husk content, g = husk content in fraction × weight
of grain used for milling, g; \( W_\text{uh} \) = Weight of unhulled grain after milling, g; \( W_\text{th} \) = Weight of grain used for milling, g; \( W_\text{fp} \) = Weight of finished product (Splits and whole dehulled grain), g; \( W_\text{br} \) = Weight of brokens, g; \( W_\text{po} \) = Weight of powder, g.

### 2.5 Enzymatic pre-treatment

The enzyme solution was prepared at the standardized proportion of all three selected enzymes. The process flowchart of enzymatic pre-treatment is given in Figure 1 for milling of pigeon pea. In case of enzymatic pre-treatment, the degumming might be due to the action of different enzymes used for pre-treatment, i.e., xylanase, pectinase and cellulase.

![Flow chart for enzymatic pre-treatment and milling of pigeon pea](image)

**Figure 1** Flow chart for enzymatic pre-treatment and milling of pigeon pea

### 2.6 Dry milling method followed as control

Generally, the dry milling method is followed throughout the Indian subcontinent for milling of pigeon pea. Hence, for the comparison of enzymatic pre-treatment, the dry milling method was taken as control. The cleaned and size graded grains were pitted through dehusking roller machine. Then, mustard oil was used for oil treatment @ 0.5 kg oil per 100 kg pigeon pea grains\[12\]. For 2 kg pigeon pea grains 10 g mustard oil was mixed and kept in a glass bottle (5 L) for 36 h for diffusion of oil. After 36 h, the distilled water was sprayed @ 100g/2 kg grain, on the grains and heaped for 12 h. Subsequently, after tempering, the grains were dried in tray dryer (Khera Instruments Pvt. Ltd., New Delhi) at 60°C up to a moisture content of 10%±0.5% (w.b.). This sequence of operation was repeated three to four times.

### 2.7 Milling of sample

Enzyme and oil treated samples of 2 kg weight having about 10%±0.5% moisture content (w.b.) were milled using laboratory scale dehusking machine. After milling, all obtained fractions were collected in polyethylene bag. Each of the samples was milled separately and care was taken to obtain all the fractions without any loss, using a cleaning brush.

### 2.8 Dehulled sample separation

The different fractions of the milled product such as whole dehulled grains, split dehulled grains, partly dehulled and unhulled grains, broken, husk and powder were separated by suitable sieves (BS sieve no. 4, 6, 18). A grain was considered completely dehulled when there was no husk adhering to it.

### 2.9 Cooking time

Pigeon pea dhal samples obtained through various enzymatic treatments were cooked in a stainless steel pan having a ratio of dhal : distilled water as 1:10. For determination of cooking time, distilled water was heated to boiling point in a 150 mL beaker and then 15 g dhal was added. During boiling, the level of water was maintained by regular addition of boiled water. Boiling was continued and samples were drawn at 1 min interval to check the cooking time by pressing between the thumb and the forefinger till no hard core is left as described by Singh et al\[13\]. Full cooking time was recorded as the time when 90% of the dhal became soft enough to masticate\[14\].

### 2.10 Sensory evaluation

Sensory evaluation was carried out on the cooked samples of enzyme treated and control samples immediately after cooking. The cooking was performed
in open pot at \((98\pm1.5){^\circ}C\) for 20 min with dhal to distilled water ratio of 1:10. The coded cooked samples were presented to the panellists. The samples were evaluated by ten untrained panellists comprising staff members of different departments of College of Agricultural Engineering and Technology. The samples were rated for six sensory attributes (colour, appearance, flavour, texture, taste and overall acceptability) on 9-point hedonic scale from 9 (like extremely) to 1 (dislike extremely)\(^{115}\).

2.11 Experimental design

The effects of four independent variables viz., enzyme concentration, incubation time, incubation temperature and pH value on cooking time were studied with variables coded as \(X_1, X_2, X_3\) and \(X_4\) respectively. The levels of parameter values were carefully chosen based on the literature available on the enzymatic hydrolysis of pigeon pea grain. Response variable, i.e., cooking time was determined for optimization of the process. Response Surface Methodology (RSM) was used for designing the experiments. A Central Composite Rotatable Design (CCRD) of 4 variables at 5 levels each with 6 centre point combinations was used\(^{116}\). Altogether, 30 combinations (including 6 replications at the centre point and single observation at other points) were chosen according to a central composite rotatable design. The coded and uncoded variable values of the design are presented in Table 2.

<table>
<thead>
<tr>
<th>Table 2 Coded and uncoded variables levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Enzyme concentration, mg/100 g dry matter</td>
</tr>
<tr>
<td>Incubation time, h</td>
</tr>
<tr>
<td>Incubation temperature, °C</td>
</tr>
<tr>
<td>Tempering water pH</td>
</tr>
</tbody>
</table>

For data analysis and optimization, the CCRD design was used to conduct experiments and the Response Surface Methodology (RSM) was applied to the experimental data using a commercial statistical package, Design Expert–version 8.0.0.6 (State-Ease Inc.\(^{117}\)). Analysis of variance (ANOVA) was calculated for fitting the model represented by Equation (1) to examine the statistical significance of the model terms. Model analysis with respect to lack-of fit test and \(R^2\) (co-efficient of determination) was done for determining adequacy of model. The coefficient of variance (CV) was calculated to find the relative dispersion of the experimental points from the prediction of the model. Response surfaces were generated and by using the same software, numerical optimization was done. The most commonly used model for optimization using response surface methodology is a second order polynomial equation\(^{118}\). The model is of the form:

\[
Y_k = b_{k0} + \sum_{i=1}^{3} b_{ki} X_i + \sum_{i=1}^{3} b_{ki} X_i^2 + \sum_{i=1, i\neq j=1}^{3} b_{ki} X_i X_j \n\]

\((k=0, 1, 2, 3, \ldots)\) (5)

where, \(Y_k\) is the response; \(b_{k0}, b_{ki}, b_{ki}\), and \(b_{ki}\) are the constant, linear, quadratic and cross-product regression coefficients, respectively and \(X_i\)'s are the coded independent variables.

2.12 Validity test

The optimum conditions obtained through statistical analysis was verified by conducting the experiment in triplicates. The average value of cooking time was considered for the validation.

3 Results and discussion

3.1 Effect of enzymatic treatment on cooking time

The response surface quadratic model implied the significant effect of selected enzymatic pre-treatments on cooking time of pigeon pea dhal. The experimental data on effect of enzyme concentration, incubation time, incubation temperature and pH value as well as their interactions on cooking time of enzyme treated pigeon pea dhal were analyzed (Table 3). The results showed that among linear effects, enzyme concentration and tempering water pH value had a significant effect on cooking time \((p<0.05)\) at 5\% level of significance. The incubation time was found to be highly significant \((p<0.01)\) at 1\% level of significance (Table 2). However, linear effects of incubation temperature and interaction effects of enzyme concentration, incubation time, incubation temperature and tempering water pH value were found to be non-significant. Quadratic effect of enzyme concentration had significant effect on cooking time \((p>0.05)\) at 5\% level of significance while incubation time and incubation temperature had a highly
significant effect on cooking time \((p<0.01)\) at 1% level of significance.

The cooking time varied from 21.5 to 24.5 min for different enzyme treated dhal samples (Table 4). The minimum cooking time was found in treatment number 5 having the combination of enzyme concentration of 50 mg/100 g dry matter, 12 h incubation time, 45°C incubation temperature and 5.5 pH of tempering water whereas, the maximum cooking time was found in experiment number 20 having the combination of enzyme concentration of 40 mg/100 g dry matter, 3 h incubation time, 50°C incubation temperature and pH 5.0. The coefficient of determination \((R^2)\) and CV\% values for cooking time were 0.9062 and 1.88, respectively.

The response surface equation for cooking time was obtained for the model of second degree in terms of coded factors as under,

\[
\text{Cooking time, min} = 22.00-0.25X_1-0.33X_2+0.083X_3-0.25X_4-0.19X_5-0.062X_6X_7+0.000X_8X_9-0.12X_2X_3-0.19X_2X_4+0.19X_3X_4+0.23X_2^2+0.29X_3^2+0.10X_4^2
\]

where, \(X_1=\) Enzyme concentration (mg/100 g dry matter); \(X_2=\) Incubation time, h; \(X_3=\) Incubation temperature, °C, and \(X_4=\) Tempering water pH

Table 3 ANOVA for effects of enzymatic treatment variables on cooking time

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean sum of squares</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>14</td>
<td>12.62</td>
<td>0.90</td>
<td>4.92*</td>
<td>0.0020</td>
<td></td>
</tr>
<tr>
<td>(X_1): Enzyme concentration</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>8.18*</td>
<td>0.0119</td>
<td></td>
</tr>
<tr>
<td>(X_2): Incubation time</td>
<td>1</td>
<td>2.67</td>
<td>2.67</td>
<td>14.55**</td>
<td>0.0017</td>
<td></td>
</tr>
<tr>
<td>(X_3): Incubation temperature</td>
<td>1</td>
<td>0.17</td>
<td>0.167</td>
<td>0.909</td>
<td>0.3555</td>
<td></td>
</tr>
<tr>
<td>(X_4): Tempering water pH</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>8.18*</td>
<td>0.0119</td>
<td></td>
</tr>
<tr>
<td>(X_1^2)</td>
<td>1</td>
<td>0.56</td>
<td>0.56</td>
<td>3.07</td>
<td>0.1003</td>
<td></td>
</tr>
<tr>
<td>(X_2^2)</td>
<td>1</td>
<td>0.062</td>
<td>0.062</td>
<td>0.34</td>
<td>0.5680</td>
<td></td>
</tr>
<tr>
<td>(X_3^2)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0000</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>(X_4^2)</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
<td>1.36</td>
<td>0.2611</td>
<td></td>
</tr>
<tr>
<td>(X_1X_2)</td>
<td>1</td>
<td>0.56</td>
<td>0.56</td>
<td>3.07</td>
<td>0.1003</td>
<td></td>
</tr>
<tr>
<td>(X_1X_3)</td>
<td>1</td>
<td>0.56</td>
<td>0.56</td>
<td>3.07</td>
<td>0.1003</td>
<td></td>
</tr>
<tr>
<td>(X_2X_3)</td>
<td>1</td>
<td>1.44</td>
<td>1.44</td>
<td>7.86*</td>
<td>0.0134</td>
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</tr>
<tr>
<td>(X_1X_2)</td>
<td>1</td>
<td>2.33</td>
<td>2.33</td>
<td>12.73**</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td>(X_2^2)</td>
<td>1</td>
<td>2.33</td>
<td>2.33</td>
<td>12.73**</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td>(X_1X_2)</td>
<td>1</td>
<td>0.297</td>
<td>0.297</td>
<td>1.62</td>
<td>0.2220</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>2.75</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>10</td>
<td>2.25</td>
<td>0.225</td>
<td>2.25</td>
<td>0.1919</td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>5</td>
<td>0.50</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation total</td>
<td>29</td>
<td>15.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R^2)</td>
<td>29</td>
<td>0.9062</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation (CV%)</td>
<td>1.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: * and ** indicate significant at 5% and 1% level of significance, respectively.

3.2 Effects of enzyme concentration and incubation time on cooking time

The effects of enzyme concentration and incubation time on cooking time were determined keeping incubation temperature and tempering water pH value constant at 50°C and 5.0, respectively which is shown in Figure 2. It could be observed that with increase in incubation time, the cooking time decreased at a particular enzyme concentration. Hydrolytic activities of enzymes lead to the conversion of complex biomolecules (polymer) into simple precursors. It also affected the relative proportion of other biomolecules which might lead to decrease the cooking time. Prolonged exposure of grain to enzymes might have increased the cooking time because of hardening effect due to combined effect of temperature and moisture. The individual effects of enzyme concentration
and incubation time on cooking time were found significant at 1% and 5% level of significance, respectively. However, their interaction effect was found non-significant.

Design-Expert® Software
Cooking time
- Design points above predicted value
- Design points below predicted value

Actual Factors
X₁ = Enzyme concentration
X₂ = Incubation time

3.3 Effects of enzyme concentration and incubation temperature on cooking time

The minimum cooking time of 21.5 min was obtained at the combination of enzyme concentration of 50 mg/100 g dry matter, 12 h incubation time, 45°C incubation temperature and 5.5 tempering water pH whereas, the maximum cooking time was found at the combination of enzyme concentration of 40 mg/100 g dry matter, 3 h incubation time, 50°C incubation temperature and 5.0 tempering water pH value. It is shown that incubation time is playing prominent role for variation in cooking time.

It could be evident that cooking time initially decreased with increase in incubation temperature and enzyme concentration and then started increasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Enzyme concentration had shown a significant effect on cooking time while incubation temperature was found to be non-significant. It could be observed that with increase in temperature, the cooking time decreased at a particular enzyme concentration. Reduction in cooking time was found because of action of enzymes like pectinase on pectic substances present in the grain. Pectic substances in combination with divalent ions of calcium and magnesium improved the cooking quality of legumes as reported by Muller\textsuperscript{[19]}. Also, the findings are in accordance with the results obtained by Singh and Rao\textsuperscript{[20]} who reported that the pectinase treatment decreased the cooking time as compared to other enzymes.

Design-Expert® Software
Cooking time
- Design points above predicted value
- Design points below predicted value

Actual Factors
X₁ = Enzyme concentration
X₂ = Incubation temperature
X₃ = Tempering water pH

Figure 2 Effects of enzyme concentration and incubation time on cooking time

Figure 3 Effects of enzyme concentration and incubation temperature on cooking time.
3.4 Effects of enzyme concentration and tempering water pH on cooking time

The effects of enzyme concentration and tempering water pH on cooking time were determined keeping incubation time and incubation temperature constant at 9 h and 50°C, respectively which is shown in Figure 4. Three dimensional responses for cooking time of enzyme treated dhal samples were generated. From these surfaces, it could be evident that cooking time initially decreased with increase in tempering water pH and enzyme concentration and then started increasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Tempering water pH value and enzyme concentration had shown a significant effect on cooking time ($p<0.05$) at 5% level of significance while interaction effect of enzyme concentration and tempering water pH value on cooking time was found to be non-significant (Table 3).

3.5 Effects of incubation time and incubation temperature on cooking time

The effects of incubation time and incubation temperature on cooking time at constant enzyme concentration (40 mg/100 g) and tempering water pH value (5.0) are shown in Figure 5. It could be evident from the figure that cooking time initially decreased with increase in incubation time and incubation temperature and then started increasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Incubation temperature had shown a non-significant effect on cooking time. Prolonged exposure of grain to enzymes increased cooking time because of hardening effect of pigeon pea grain due to combined effect of temperature and moisture. Combined effect of temperature and moisture means hydrothermal treatment on grain which increases hardness of grain.
with increase in tempering water pH value, the cooking time decreased at a particular incubation time. From these surfaces, it could be evident that cooking time initially decreased with increase in tempering water pH value and incubation time and then started increasing.

**Effects of incubation time and tempering water pH on cooking time**

The effects of incubation temperature and tempering water pH value on cooking time at constant enzyme concentration (40 mg/100 g) and incubation time (9 h) are shown in Figure 7. It could be observed that with increase in tempering water pH value, the cooking time decreased at a particular incubation temperature. Tempering water pH value had shown a significant effect on cooking time and a sharp decrease in cooking time up to a certain pH value. However, incubation temperature and interaction of these two factors were found to be non-significant (Table 3).

### 3.8 Optimization of enzymatic treatment variables

Software Design Expert version 8.0.0.6 was used for the optimization of responses. A stationary point, i.e., a point at which the slope of the response surface was zero in all directions was calculated by partially differentiating the model with respect to each variable, equating these derivatives to zero and simultaneously solving the resulting equations. The optimum value of enzymatic hydrolysis pre-treatment was evaluated using equation (2). The response surface quadratic model optimized the pre-treatment as enzyme concentration of 37.80 mg/100 g dry matter, incubation time 8.69 h, incubation temperature 48.48°C and tempering water pH 5.49 which gave the predicted value of cooking time 21.91 min. The optimum values of different variables for enzymatic treatment were found within the range considered in the study.
3.9  Validity of the model

The performance of this model was also verified by conducting an experiment for the validation. In order to validate the optimum conditions of enzymatic pre-treatment variables, the experiment was conducted in triplicate at derived conditions. This was experimentally verified in the laboratory and observed value of cooking time was found to be 21.50 min. The predicted values of cooking time obtained from equation showed 2.19% deviation from the experimental values. From the results, it could be revealed that the experimental value was very close to the predicted value which confirmed the optimum conditions.

3.10 Comparison of enzymatic and oil pre-treatment

The cooking time of oil treated (control) sample was found 26.8 min while the observed value of cooking time at the optimum conditions of enzymatic pre-treatment variables was 21.50 min. Hence, there was an increase in decrease in cooking time 19.77% over oil treated sample.

The cooking time of enzyme treated pigeon pea dhal was found 21.5 min which indicated 5.3 min less time in enzyme treated dhal in comparison to oil treated dhal. These findings are in accordance with the results obtained by Saxena and Srivastava[11] who reported that the enzyme treated dhal took 3 min less time in cooking over control.

3.11 Sensory attributes of the optimized enzymatic and oil treated (control) cooked blended dhal

Sensory evaluation indicated that both the treatment got almost equal ratings in terms of taste and flavour while there was little variation in colour and textural ratings (Table 5).

Table 5  Effects of enzymatic and oil treatment on sensory quality of cooked blended dhal

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Colour</th>
<th>Texture</th>
<th>Taste</th>
<th>Flavour</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic treatment</td>
<td>7.5</td>
<td>8.5</td>
<td>8.25</td>
<td>7.0</td>
<td>7.8</td>
</tr>
<tr>
<td>Oil treatment</td>
<td>7.0</td>
<td>7.5</td>
<td>8.00</td>
<td>7.0</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Colour variation in both the treatments might be due to colour pigment of some intact husk on dhal found during oil treatment. Variation in textural ratings might be due to higher cooking time required in case of oil treatment. These results are in agreement with the results reported by Singh and Rao[15]. The sensory evaluation indicated that the dhal obtained through enzymatic pre-treatment had higher value of overall acceptability as compared to control sample.

4  Conclusions

For enzymatic pre-treatment, the enzyme solution having 2:1:1 proportion of xylanase, pectinase and cellulase enzymes should be prepared using tempering water pH value of 5.49. The enzyme solution should be applied at the rate of 37.80 mg/100 g of dry pigeon pea grain. The enzyme treated pigeon pea grains should be kept at 48.5°C incubation temperature for 8.69 h incubation time. The observed value of cooking time at the suggested conditions of enzymatic pre-treatment variables was 21.5 min.

Mathematical model predicted a minimum cooking time 21.91 min at optimum enzyme concentration of 37.80 mg/100 g dry matter, incubation time 8.69 min., incubation temperature 48.5°C and tempering water pH value of 5.49. There was a decrease in cooking time by 19.77% over oil treated sample. The sensory evaluation indicated that the dhal obtained through enzymatic pre-treatment had higher value of overall acceptability as compared to control samples.

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