Dhal recovery from enzyme pretreated pigeon pea cultivar GJP1

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Abstract: GJP1 variety is a newly developed variety of pigeon pea. It gives higher seed yield as compared to other varieties. Pigeon pea is mostly consumed in the form of splits hence, it is necessary to verify its hulling efficiency and utmost dhal recovery. Pre-milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion and better dhal recovery. Xylanase, pectinase and cellulose enzymes were used to evaluate the milling properties of pigeon pea grains. Efforts were made to evaluate the effect of enzymatic parameters, i.e., enzyme concentration (20-50 mg 100 g^{-1} dry matter), incubation time (4-12 h), incubation temperature (35°C-55°C) and tempering water pH (4.0-6.0) on hulling efficiency were optimized using response surface methodology. A quadratic model satisfactorily described the hulling efficiency with a high value for the coefficient of determination R^2 (0.92). It predicted a maximum hulling efficiency of 84.24% at enzyme concentration, 28.79 mg 100 g^{-1} dry matter, incubation time, 7.46 h, incubation temperature, 45°C and tempering water, pH 4.96. The results of the predicted optimum conditions were validated experimentally with three replications. Hulling efficiency at optimum condition was observed to be 82.80% and showed 1.44% deviation from the predicted valued. Results of hulling efficiency were also compared with traditional oil pretreated method (76.63%). It revealed that hulling efficiency of enzyme pretreated pigeon pea could be increased 8.10% compared to the oil pretreated method.

Keywords: pigeon pea, enzyme, dhal recovery, pretreatment, hulling efficiency

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1 Introduction

In the Indian subcontinent pigeon pea is predominantly consumed in the form of dhal and conversion of whole seed into dhal is a big industry in the country. For commercial purposes, big machines are used for dehulling while in rural areas, dehulling is done by using traditional grinding stones called chakki or quern. Since the cotyledons of pigeon pea are attached tightly with seed coat by gums, the processing primarily involves loosening of husk followed by dehusking and splitting of the two cotyledons. Therefore, pigeon pea dehulling is not only difficult but also a specialized function when compared with other legumes. Losses of seed mass during the process of dehulling are a common event. Excluding the husk which accounts for about 15%, the dhal recovery in around 60% by chakki and around 70% by machines (Singh and Jambunathan, 1981). This means even by using advanced technology about 15%-17% of grain mass is lost. By using chakki such losses shoot up to 20%-25%.

Reddy et al. (1979) studied the protein deposition pattern in pigeon pea seed and reported that the outer layers of the cotyledons are richer in protein in comparison to inner layers of seed. From nutrition point of view, this is a matter of concern since dehulling not only removes protine-rich germ but also the outer layers of the cotyledons where relatively more protein constituents are housed. Fortunately, the protein quality in terms of amino acids is not adversely affected by dehulling. Singh and Jambunathan (1990) further reported that dehulling also removers about 20% calcium and 30% iron. To preserve the nutritive value of pigeon

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pea seed and minimizing the nutrient losses during dehulling it is essential that more efficient dehulling technology is developed and transferred to rural areas where by and large milling is still carried out by inefficient old-age techniques. According to Kurien (1981) under controlled conditions the dhal yield achieves the maximum efficiency of 80%-84% but at commercial level the recovery remains around 70%. He also reported large varietal difference (72%-82%) for dhal yield. Therefore, it can be assumed that with a combination of a superior variety and an efficient pigeon pea processing technology, the nutrient losses can be minimized.

Several previous studies reported that the husk of grain adhered to the cotyledons due to the presence of calactomonus disaccharide, glucoronai acid and glycol protein (Kurien and Parpia, 1968). For adherence of husk to the cotyledons, arabinogalactan type polysaccharide was found responsible, which is gummy and hygroscopic in nature (Swamy et al., 1991). The presence of these complex carbohydrates makes the dehulling of pigeonpea a difficult process. Therefore, milling of pulses without pre-treatment results in low dhal recovery. Pre-milling treatments play an important role in improving dhal recovery by loosening husk from cotyledons (Saxena, 1999). Different pre-treatments viz., water soaking, water spray with oil treatment, sodium bicarbonate treatment and enzyme treatment except sodium bicarbonate treatment caused a significant loss in protein content of cotyledons over untreated samples (Phirke and Bhole, 2000). The effects of chemical treatment on husk removal of pigeonpea grain using aqueous solutions of calcium hydroxide, sodium hydroxide and sodium bicarbonate was observed and among them sodium bicarbonate solution was the most effective for dhal recovery (Saxena et al., 1981). Sharanagouda et al. (2011) reported that Gulyal variety treated with mustard oil recorded maximum hulling efficiency (79.4%) and finished product (68.8%) when compared to a Maruti and Asha variety. However, acetic acid treatment recorded higher hulling efficiency (76.5%) for Maruti followed by Asha (56.9%). Krishnamurthy et al. (1972) reported that 'sirka' can be used in place of oil in Arhar milling. It was observed that the recovery in this process was same as in case of oil application. Enzyme pre-treatment resulted 13.81% higher recovery of dhal as compared to oil treatment for BDN-2 variety of pigeon pea (Sangani et al., 2014). The dal recovery and milling efficiency at optimized enzymatic hydrolysis parameters were 76.60% and 96.19%, respectively (Murmurkar et al., 2016). The partial disruption/degradation of non-starch polysaccharides and/or proteins of mucilage, which is present in between hulls and cotyledon by enzymes, has facilitated the improvement in the dehulling properties of hard-to-dehull legumes. Protease pre-treatment of green gram and black gram resulted in higher yield of dehulled kernels. Xylanase pre-treatment was very effective in improving the dehulling properties of horse gram compared to protease, which resulted in the yield of more undehulled kernels and fines. Protease pre-treatment produced more dehulled kernels in red gram than xylanase. It is also evident that the enzyme dehulling pre-treatments not only increased the dehulling efficiency, but also reduced the amount of powder and fines formed (Sreerama et al., 2009). Enzyme treated target grains were found to utilize less time for dehusking as compared to water treated grains used in conventional milling. The enzyme treated grains were found to be brighter in colour in comparison to untreated grains. Additionally, there were changes observed in the amount of broken grains and powder formation i.e., after processing of the grains, the powder formation and number of broken grains reduced significantly which bolsters the overall reason for application of enzymes for dehusking (Chandini et al., 2016).

Keeping in view of above newly developed variety of pigeon pea i.e. GJP1 was selected for the study with an objective to standardize the enzymatic pre-milling treatments to increase the dhal recovery.

2 Material and methods

2.1 Selection of variety

Amongst different varieties of pigeon pea being cultivated in Gujarat, the GJP1 variety is newly developed by Junagadh Agricultural University in 2014. This variety produced higher seed yield over BDN–2, ICPL 87119, and AGT-2 respectively (Anon, 2014). This variety is medium late (176 days) in maturing. GJP 1 is also found moderately resistant to Wilt and SMD disease.

The seed of this variety is bold in sized with white color. It is necessary to test its hulling efficiency for milling purpose.

2.2 Dehusking machine

The laboratory scale dehusking machine based on CIAE dhal mill design and fabricated by Bharodia (Salve et al., 2008). With overall dimensions of 600 mm × 620 mm × 935 mm, capacity of 85 kg h^{-1} 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 1420 rpm and 64 kg h^{-1}, respectively.

2.3 Selection of enzymes

The selection of enzymes was made based on 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 (Saxena and Srivastava, 1998). 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 cellulose 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 tempering water pH constant based on the technical specifications of the products provided by manufacturer (Table 1).

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 (Shanta et al., 1978).

$$Husk\ removed(HR),\ \% = \frac{Husk\ Removed\ during\ dehusking}{Total\ husk\ content} \times 100 \quad (1)$$

$$Coefficient\ of\ hulling\ (Ch) = 1 - (Weight\ of\ unhulled\ grain\ after\ milling)/(Weight\ of\ unhulled\ grain\ used\ ffor\ milling) \quad (2)$$

$$Coefficient\ of\ wholeness\ of\ kernel\ (Cwk) = (Weight\ of\ finished\ product\ (Split\ and\ whole\ dehulled\ grain))/(Weight\ of\ finished\ product + Weight\ of\ brokens + Weight\ of\ powder) \quad (3)$$

$$Hulling\ efficiency = Ch \times Cwk \times 100 \quad (4)$$

Table 1 Technical specifications of enzymes supplied by the manufacturer

Specification	Enzymes		
	Xylanase	Pectinase	Cellulase
Appearance	Off white	Off white	Light brown
Solubility	Soluble in water	Soluble in water	Soluble in water
Storage condition, °C	2-8	2-8	2-8
Optimum temperature range, °C	30-60	45-50	40-50
Optimum tempering water pH range	4.5-5.5	5.0-5.5	4.0-5.0
Enzyme activity	12.5 u mg^{-1}	---	≥10 u mg^{-1}

2.5 Enzymatic pre-treatment

The enzyme solution was prepared at the standardized proportion of all three selected enzymes. In this enzymatic pre-treatment, the degumming might be due to the action of different enzymes used for pre-treatment, i.e., xylanase, pectinase and cellulase.

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 oilwas used for oil treatment: 0.5 kg oil per 100 kg pigeon pea grains (Saxena and Srivastava 1998). 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 100 g 2 kg^{-1} 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 or four times.

2.7 Milling of sample

Enzyme and oil treated samples of 1 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 Experimental design

The effects of four independent variables viz., enzyme concentration, incubation time, incubation temperature and tempering water pH value on cooking time were studied with variables coded as X1, X2, X3 and X4 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 four variables at five levels each with six centre point combinations was used (Khuri and Cornell, 1987). Altogether, 30 combinations (including six replications at the centre point and a 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 2 Coded and uncoded variables levels

Variables		Coded variables				
		–2	–1	0	+1	+2
Enzyme concentration mg 100 g^{-1} dry matter	(X1)	20	27.5	35	42.5	50
Incubation time, h	(X2)	4	6	8	10	12
Incubation temperature, °C	(X3)	35	40	45	50	55
Tempering water pH	(X4)	4.0	4.5	5.0	5.5	6.0

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.2009). 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 (Bas and Boyaci, 2007). The model is of the form:

$$Yk = bk0 + \sum\_(i=1)\text{^}3\text{▒}〖bki\ Xi + \sum\_(i\neq j=1)\text{^}3\text{▒}〖bkij\ Xi\ Xj〗 + \sum\_(i=1)\text{^}3\text{▒}〖bkii\ X\_i\text{^}2+\varepsilon〗〗 \quad (k=0, 1, 2, 3\ldots.) \tag{5}$$

where, Yk is the response; $bk0$, bki, $bkij$, $bkii$ and ε are the constant, linear, and quadratic cross-product regression coefficients, random error respectively and Xi's are the coded independent variables.

2.10 Validity test

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

3 Results and discussion

3.1 Effect of enzymatic treatment on hulling efficiency

The response surface quadratic model implied the significant effect of selected enzymatic pre-treatments on hulling efficiency of pigeon pea. The experimental data on effect of enzyme concentration, incubation time, incubation temperature and tempering water pH value as well as their interactions on hulling efficiency of enzyme treated pigeon pea dhal were analyzed (Table 3). Results showed that among linear effects of enzyme concentration and incubation temperature had non-significant effect on hulling efficiency whereas incubation time and tempering water pH had a significant

effect at 5% level of significance. Interaction effects of enzyme concentration with any of other parameter were found to be non-significant but interaction effect of incubation temperature with incubation time as well as with tempering water pH had a significant effect with 1% level of significance (p<0.01). Interaction effect of incubation time with tempering water pH had also significant effect with 1% level of significance (p<0.01). Quadratic effect of enzymatic concentration, incubation time and tempering water pH had non-significant effect on hulling efficiency whereas quadratic effect of incubation temperature had a significant effect at 1% level of significance (p<0.01). Sangani et al. (2014) reported the quadratic effect of enzyme concentration, incubation temperature and tempering water pH having significant effect on hulling efficiency. Murmurkar et al. (2016) reported quadratic effect of enzyme concentration, incubation temperature and incubation time having significant effect on hulling efficiency.

Table 3 ANOVA for effects of enzymatic treatment variables on hulling efficiency

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1379.26	14	98.52	11.98**	<0.0001
X_1-Enzyme Concentration	5.40	1	5.40	0.66	0.4305
X_2-Incubation Time	39.99	1	39.99	4.86*	0.0434
X_3-Incubation Temperature	19.40	1	19.40	2.36	0.1453
X_4-pH	65.94	1	65.94	8.02*	0.0126
X_1X_2	4.75	1	4.75	0.58	0.4588
X_1X_3	7.00	1	7.00	0.85	0.3709
X_1X_4	1.31	1	1.31	0.16	0.6953
X_2X_3	201.07	1	201.07	24.46**	0.0002
X_2X_4	272.91	1	272.91	33.20**	<0.0001
X_3X_4	194.46	1	194.46	23.65**	0.0002
X_1^2	3.98	1	3.98	0.48	0.4973
X_2^2	4.34	1	4.34	0.53	0.4785
X_3^2	524.60	1	524.60	63.81**	<0.0001
X_4^2	0.91	1	0.91	0.11	0.7446
Residual	123.31	15	8.22		
Lack of Fit	80.70	10	8.07	0.95	0.5618
Pure Error	42.61	5	8.52		
Correlation Total	1502.58	29			
R^2	0.9172				
Coefficient of variation	3.53				

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

The hulling efficiency varied from 62.12 to 86.47 percent (Table 4). The maximum hulling efficiency was found in treatment having the combination of enzyme concentration 27.5%, incubation temperature 50°C, incubation time 10 hrs and tempering water pH 5.5 while, minimum hulling efficiency was found in the treatment having the combination of enzyme concentration 35%, incubation temperature 35°C, incubation time 8 hrs and tempering water pH 5.0. This showed that enzyme concentration, incubation time, incubation temperature and tempering water pH played prominent role on hulling efficiency.

Table 4 Effect of enzymatic treatment variables on hulling efficiency

Treat No.	Enzymatic treatment variables				
	Enzyme concentration (mg 100 g^{-1} dry sample)	Incubation Time (h)	Incubation Temp (°C)	Tempering water pH	Hulling Efficiency (%)
	Traditional method				76.63
1	42.5	10	50	5.5	86.45
2	27.5	10	50	5.5	86.47
3	42.5	6	50	5.5	86.45
4	27.5	6	50	5.5	86.33
5	42.5	10	40	5.5	74.64
6	27.5	10	40	5.5	73.33
7	42.5	6	40	5.5	84.63
8	27.5	6	40	5.5	82.80
9	42.5	10	50	4.5	86.40
10	27.5	10	50	4.5	85.50
11	42.5	6	50	4.5	62.31
12	27.5	6	50	4.5	67.65
13	42.5	10	40	4.5	84.48
14	27.5	10	40	4.5	81.48
15	42.5	6	40	4.5	80.64
16	27.5	6	40	4.5	80.64
17	50	8	45	5	78.96
18	20	8	45	5	85.50
19	35	12	45	5	86.30
20	35	4	45	5	84.39
21	35	8	55	5	70.40
22	35	8	35	5	62.12
23	35	8	45	6	86.40
24	35	8	45	4	82.56
25	35	8	45	5	86.32
26	35	8	45	5	86.40
27	35	8	45	5	86.33
28	35	8	45	5	80.64
29	35	8	45	5	80.75
30	35	8	45	5	86.37

R^2 and CV percent value for hulling efficiency was 0.97 and 3.53 percent respectively. The response surface equation of second order was obtained in terms of coded factors to predict the variation in hulling efficiency due to

enzyme pre-treatment on pigeon pea processing with varying levels of processing parameters under,

$$\textit{Hulling efficiency}\ (\%)=84.46-0.47X_1+1.29X_2+0.907X_3+1.66X_4+0.55X_1X_2+0.66X_1X_3+0.29X_1X_4+3.55X_2X_3-4.13X_2X_4+3.49X_3X_4-0.38X_1^2+0.40X_2^2-4.37X_3^2-0.18X_4^2 \quad (6)$$

where, X_1, X_2, X_3 and X_4 are the coded factors of enzyme concentration, incubation time, incubation temperature and tempering water pH, respectively.

3.2 Effect of enzyme concentration and incubation time on hulling efficiency

The effect of enzyme concentration and incubation time on hulling efficiency was determined keeping incubation temperature and tempering water pH at 45°C and 5.0 respectively. Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples was generated (Figure 1a). It could be observed that hulling efficiency was increased with an increase of enzyme concentration up to 35.20 mg 100 g^{-1} sample and incubation time up to 7.86 h, respectively. This interaction of enzyme concentration and incubation time was proposed to increase the hulling efficiency up to 85.49%. However, with further increase in enzyme concentration and incubation time, the hulling efficiency was decreased. This may indicate the existence of optimum levels of hydrolysis parameters within the selected range. The reduction in enzymatic activity at higher enzyme concentration might be due to saturation of active sites of enzymes with substrate leading to lower hulling efficiency. However, the effect of enzyme concentration on hulling efficiency was found to be non-significant. Higher incubation time might have produced inhibitor substances for enzyme action resulting in lower hulling efficiency. Prolonged exposure of grain to enzymes may have decreased the hulling efficiency because of hardening effect due to combined effect of temperature and moisture (Sangani et al., 2014; Murmurkar et al., 2016). The individual effect of enzyme concentration was found nonsignificant. The results were confirmed by Sangani et al. (2014) reporting that the enzyme concentration effect was nonsignificant for hulling efficiency but Murmurkar et al. (2016) reported the highly significant effect of enzyme concentration on hulling efficiency. Individual effect of incubation time on hulling efficiency was found significant at 5% level of significance. These findings were confirmed by Murmurkar et al. (2016) but interaction of enzyme concentration and incubation time has a nonsignificant effect on hulling efficiency.

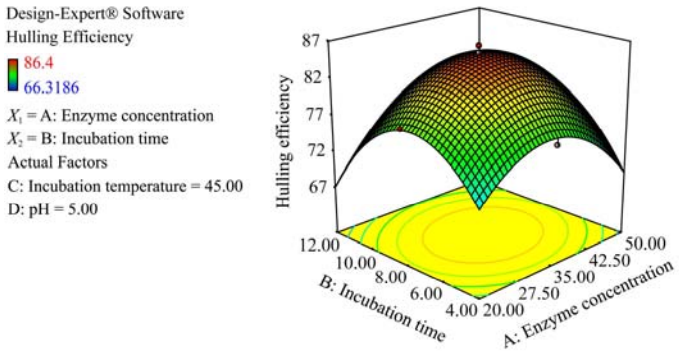
3.3 Effect of enzyme concentration and incubation temperature on hulling efficiency

The effect of enzyme concentration and incubation temperature on hulling efficiency was determined keeping incubation time and tempering water pH at 8 h and 5.0 respectively. Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples were generated. Figure 1b shows three-dimensional response surface plot for hulling efficiency, which indicated that there was an increase in hulling efficiency with an increase in enzyme concentration up to 35.23 mg 100 g^{-1} sample and incubation temperature up to 44.92°C. The hulling efficiency was expected to be increased up to 85.49% at this combination of enzyme concentration and temperature. The hulling efficiency was found to be decreased with further increase in enzyme concentration and temperature. Individual effect of incubation temperature was also nonsignificant. This finding was contradiction to the findings of Sangani et al. (2014) and Murmurkar et al. (2016). Interaction effect of enzyme concentration and temperature was nonsignificant. The results were confirmed by Sangani et al. (2014). The reduction in enzymatic activity at above optimum incubation temperature was due to denaturing of enzyme, resulting in the reduction in hulling efficiency. It also confirmed the facts that maximum enzymatic reaction occurred at optimum enzyme concentration and temperature levels.

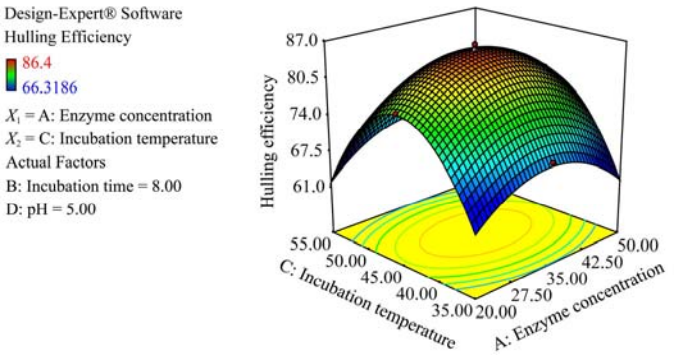
3.4 Effect of enzyme concentration and tempering water pH on hulling efficiency

The effect of enzyme concentration and tempering water pH on hulling efficiency was determined keeping incubation period and incubation temperature at 8 h and 45°C respectively. Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples was generated. Figure 1c shows the response surface plot for hulling efficiency, which indicated that there was an increase in hulling efficiency with an increase in enzyme concentration up to 35.30 mg 100 g^{-1} sample and

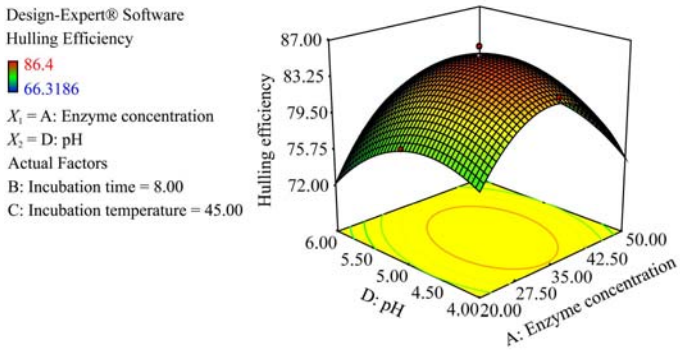
tempering water pH up to 4.88. The hulling efficiency was expected to be increased up to 85.53% at this combination of enzyme concentration and tempering water pH. The hulling efficiency was found to be decreased with further increase in enzyme concentration and tempering water pH thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Individual effect of tempering water pH had a significant effect at 5% level of significance. Interaction of enzyme concentration and tempering water pH had a nonsignificant effect on hulling efficiency. The results were confirmed by Sangani et al. (2014). It could be observed that with increase in tempering water pH, the hulling efficiency increased at a particular enzyme concentration. The reduction in enzyme activity at above optimum tempering water pH was due to denaturing of enzymes, resulting in a decrease in the hulling efficiency.



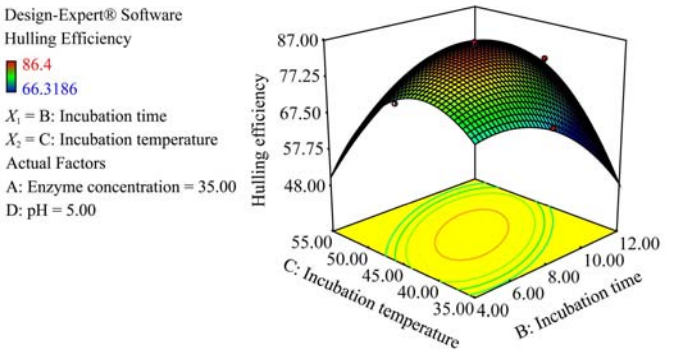
a. Effect of enzyme concentration and incubation time
(enzyme concentration (mg 100 g^{-1} dry matter) and incubation time (h))



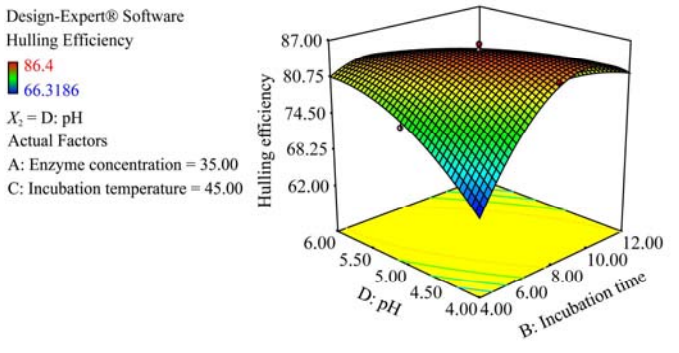
b. Effect of enzyme concentration and incubation temperature
(enzyme concentration (mg 100 g^{-1} dry matter) and incubation temperature (°C))



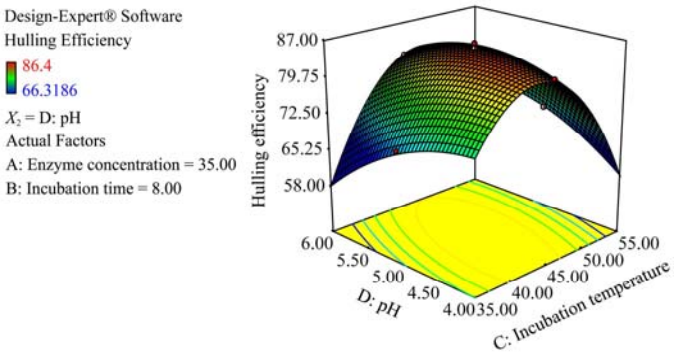
c. Effect of enzyme concentration and tempering water pH
(enzyme concentration (mg 100 g^{-1} dry matter) and tempering water pH)



d. Effect of incubation time and incubation temperature
(incubation time (h) and incubation temperature (°C))



e. Effect of incubation time and tempering water pH
(incubation time (h) and tempering water pH)



f. Effect of incubation temperature and tempering water pH
(incubation temperature (°C) and tempering water pH)

Figure 1 The effect of voltage of various parameters on hulling efficiency of pigeon pea

3.5 Effect of incubation time and incubation temperature on hulling efficiency

The effect of incubation time and incubation temperature on hulling efficiency was determined keeping enzyme concentration and tempering water pH at (35 mg 100 g^{-1} sample) and 5.0 respectively.

Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples was generated. Figure 1d shows the response surface plot for hulling efficiency, which indicated that there was an increase in hulling efficiency with an increase in incubation time up to 7.90 h and incubation temperature up to 44.98°C. The hulling efficiency was expected to be increased up to 85.47% at this combination of incubation time and incubation temperature. The hulling efficiency was found to be decreased with further increase in incubation time and incubation temperature. Interaction of incubation time and incubation temperature had significance effect at 1% level of significance on hulling efficiency. This finding was contradiction to the finding of Sangani et al. (2014). The reduction in enzyme activity at above optimum incubation temperature would denature the enzymes, resulting in a decrease in the hulling efficiency.

3.6 Effect of incubation time and tempering water pH on hulling efficiency

The effect of incubation period and tempering water pH on hulling efficiency was determined keeping enzyme concentration and incubation temperature at (35 mg 100 g^{-1} sample) and 45^{o}C respectively. Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples was generated. Figure 1e shows the response surface plot for hulling efficiency, which indicated that there was an increase in hulling efficiency with an increase in incubation time up to 8.14 h and tempering water pH up to 4.83. The hulling efficiency was expected to be increased up to 85.56% at this combination of incubation time and tempering water pH. The hulling efficiency was found to be decreased with further incubation time and tempering water pH. Interaction of incubation time and tempering water pH had a significant effect at 1% level of significance on hulling efficiency. This finding was contradiction to the finding of Sangani et al. (2014).

3.7 Effect of incubation temperature and tempering water pH on hulling efficiency

The effect of incubation temperature and tempering water pH on hulling efficiency was determined keeping enzyme concentration and incubation period at (35 mg 100 g^{-1} sample) and 8 h respectively. Three-dimensional response surface plot for hulling efficiency of enzyme pretreated samples was generated. Figure 1f shows the response surface plot for hulling efficiency, which indicated that there was an increase in hulling efficiency with an increase in incubation temperature up to 44.54^{o}C and tempering water pH up to 4.80. The hulling efficiency was expected to be increased up to 85.56% at this combination of incubation temperature and tempering water pH. The hulling efficiency was found to be decreased with further increase in incubation temperature and tempering water pH. Individual effect of incubation temperature had non-significant effect whereas tempering water pH had a significant effect at 5% level of significance. Interaction of incubation temperature and tempering water pH had a significant effect at 1% level of significance on hulling efficiency. This finding was contradiction to the finding of Sangani et al. (2014).

3.8 Optimization of process variables

The optimum condition to produce pigeon pea dhal was determined by the numerical optimization technique, using Design Expert version 7.0.0 (Trial version; State-Ease Inc., Minneapolis, MN, USA). 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 zero and simultaneously solving the resulting equations. The optimum values of enzymatic hydrolysis pre-treatment were evaluated using Equation 6. The multiple regression package was used for this purpose. The response surface quadratic model optimized the pre-treatment as enzyme concentration of 28.79 mg 100 g^{-1} dry matter, incubation time 7.46 h, incubation temperature 44.97^{o}C (≈45^{o}C) and tempering water pH 4.96 which gave the predicted values of hulling efficiency 84.24%. The main criteria applied for constraints optimization in the study were: (a) enzyme concentration: minimum, (b) hulling efficiency: maximum. The constraints, criteria and output for numerical optimization of pigeon pea dhal production are given in Table 5.

It may be mentioned that the optimum values of different variables for enzymatic pre-treatment were

within the range considered in the study.

The hulling efficiency of oil treated (control) sample was found 76.63% while the observed value of hulling efficiency of enzymatic treatment sample at optimum condition was 82.80%. Hence, there was an increase in hulling efficiency of 8.10% over oil treated sample.

Table 5 Constraints, criteria and output for numerical optimization of pigeon pea dhal production

Variables						
Constraint		Goal		Importance		Optimum value
Enzyme concentration (%)		Minimize		3		28.79
Incubation time (h)		In the range		3		7.46
Incubation temperature (°C)		In the range		3		44.97
Tempering water pH		In the range		3		4.96
Responses						
Constraint	Goal	Importance	Predicted value	Experimental value	Deviation (%)	Random error
Hulling efficiency (%)	Maximize	5	84.24	82.80	1.44	1.71%
Desirability	--	--	0.846	-	-	

4 Conclusions

For enzymatic pre-treatment, mathematical model predicted a maximum dehulling efficiency of 84.24% at optimum enzyme concentration of 28.79 mg 100 g^{-1} dry matter, incubation time 7.46 h., incubation temperature 45^{o}C and tempering water pH 4.96. Dehulling efficiency at optimum condition was experimentally observed to be 82.80% and were close to the predict value.

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