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EFFECT OF ENZYME PRE-TREATMENTS ON MILLING OF PIGEONPEA

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Abstract: Xylanase, pectinase and cellulose pre-treatments were used to evaluate the milling properties of pigeon pea grains. Efforts were made to evaluate the suitability of enzyme treatment for milling of pigeon pea with different concentrations (35 mg/100 g dm to 55 mg/100 g dm), incubation temperatures (35 to 55^{O}C) and incubation periods (4 to 12 h). The application of cellulase, xylanase and pectinase, was found to be effective in improving the milling properties of pigeon pea grains with increased dal recovery. Enzyme treatments were found to have no adverse effect on cooking properties of pigeon pea grains. The dal recovery and milling efficiency at optimized independent parameters were 76.60 and 96.19%, respectively.

Introduction

India is the largest producer of pulses in the world. India produced 18.45 million tones of pulses in 2012-13 (www.indiastat.com) Pulses are chief and cheap source of body-building proteins particularly for vegetarians and for poor because animal proteins are beyond their reach. The Increase in population and stagnation of the pulse production in India has resulted in the reduction of its per capita availability from 27.5 kg in 1959 to merely 14.94 kg in 2003-2004 as compared to recommended annual requirement of 23.5 kg for balanced diet (Phirke 1993). The production 15.24 million tones, which is the 4/5th share in the world and 1/5th share in the total pulse production in the country. Maharashtra prouduces the maximum level of pigeonpea in India accounting to 700000 tons. (Anonymous, 2013)

In India, about 80% of the pulse production is consumed in the form of dal or powder and remaining 20% as the whole seed and other forms (Chacko et al. 2001; Mangaraj et al. 2005). The whole pulses are milled into splits (dal) by various methods/processes. The dal recovery varies from 60 to 75%, depending upon the type of pulses and techniques adopted by the millers such as methods of pretreatment and milling machinery used (Sahay and Bisht 1988; Mangaraj et al. 2005). Generally, the husk is tightly attached to the cotyledons in pulses (Chakravarty, 1988). In most pulses, husks are attached with cotyledons through a layer of gums (Kurien and parpia, 1968). Pigeonpea is considered most difficult grain for removing

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the seed coats due to gum and mucilage present in it. The dehulling of pigeonpea grain involves two steps; pretreatment of grain for loosening the seed coat and its removal from the cotyledons, called dehulling. In order to loosen the husk from cotyledon, various pretreatments are employed. Hence, a pretreatment of pulse grain for loosening of the husk prior to milling is desirable as it increases the recovery of dal (Sahay et al. 1985; Mangaraj et al. 2004). Premilling treatment is very important operation in dal milling of difficult to mill pulses as it is aimed at loosening of husk. For pretreatment of pigeonpea commonly used processes involves oils, chemical and heat. Oil and water is most commonly used premilling ptreatment. It is estimated that about 199.02 million kg edible oil is required every year in pulses milling industry.

The enzyme premilling treatment may help to reduce the number of passes during milling. The study was therefore conducted to evaluate the suitability of enzyme pretreatments on milling of pigeonpea and to optimize the input parameters for better milling characteristics**.**

Materials and methods

Procurement of raw material

The pigeon pea grains were procured from Central Research Station (CRS), Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. These grains were given different pre-treatments for loosening of seed coat in order to have better dehulling.

Procurement of enzyme

The industrial enzymes were procured from private firm M/s Anthem Cellutions, Bangalore and used for experimentation.

Preparation of sample

Cleaning grading

The procured pigeon pea grains were cleaned and graded by PKV cleaner grader to remove all foreign matter such as dust, dirt, stones, chaff, immature grains, etc. and to obtain uniform size grains for the experimentation. The grains were then scarified (pitted) in PKV mini dal mill (first pass).

Moisture content

The initial moisture content of pigeon pea grain sample was determined by hot air-oven method. (Sahay and Singh, 1994). Moisture content of sample was determined based on drop in weight from initial weight of sample by using following formula.

$$\underset{(\%,\ w.b.)}{\text{Moisture content}} = \frac{\text{Initial wt. of sample - Oven dried wt. of sample}}{\text{Initial weight of sample}} \times 100 \quad \text{---- (1)}$$

Seed coat content

The seed coat content in whole grain was determined by soaking 5 g of pigeon pea grains in distilled water (2 h at 50 ^{o}C). The seed coat were then separated manually from the cotyledons, dried in hot air oven at 100 ± 5 ^{o}C up to initial moisture content of 10 % (w.b.) (Bharodia, 2004). The seed coat content in % was calculated using following formula.

Seed coat content, % SC = 100(Wh-Wp) / Wh … (2)

Where,

Wh = Weight of whole grain, (g)

Wp = Weight of pearled grain, (g)

Treatments

The experiments on dehulling of pigeon pea were carried out by using various pre-treatments viz., enzymic treatment and water soaking treatments as below.

Enzyme treatment

Independent variables

i. Concentration of enzyme (mg/ 100 g dm) - 35, 45 and 55 (3 levels)

ii. Incubation temperature (^{o}C) - 35, 45 and 55 (3 levels)

iii. Incubation period (h) - 4, 8 and 12 (3 levels)

Water soaking treatment

In the water soaking treatment pigeon pea grains were no soaked and soaked in water for six different durations i.e. 0 min, 5 min, 10 min, 15 min, 20 min and 25 min.

After their respective completion of soaking time, the grains were heaped for 30 min and they were removed and kept for drying in tray dryer till moisture content becomes 10 % (w.b). The samples of dried pigeon pea grains were then dehulled in PKV Mini Dal Mil.

Dependent variables

i. Dehulling Efficiency (DE)

ii. Dal Recovery

The dry milling method (oil treatment) was used as control.

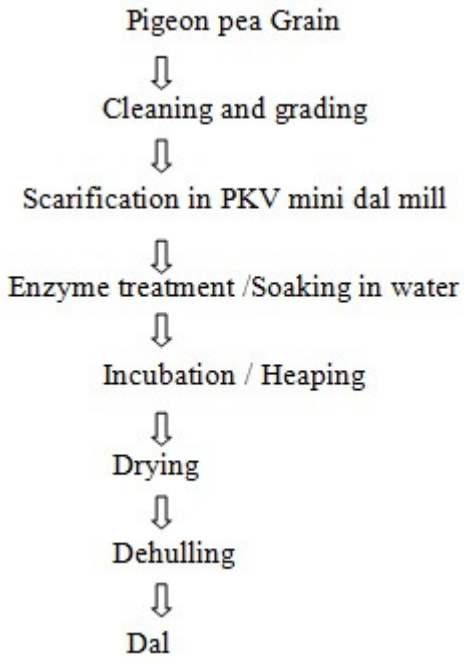
Milling of treated sample

The PKV Mini dal mill was used as dehulling device to study the effect of various treatments on dehulling of pigeon pea grains. The dehulled samples were collected separately and analyzed manually for different fractions such as: Dehulled intact grain, Dehulled splits,

Undehulled intact grain, Undehulled splits, Brokens, Husk, Mixture of fine husk and powder. These fractions were used to determine various response parameters as Dehulling efficiency (DE), Dal recovery (DR), Dehulling index (DI) and Degree of Dehulling (DD).

The optimum operating speed of the machine was 1420 rpm and the feed rate during the first pass (scarified) and second pass was kept constant at 150 and 100 kg/h, respectively. The dry milling method was used during the milling process.

The process flowchart of enzyme pre-treatment is given for milling of pigeon pea.



Process flow chart for pigeon pea dehulling

Preparation of enzyme solution

The amount of the water having 5 pH was used (2100 ml for 10 kg grain) and required amount of enzymes were weighed and dissolved in water (Sangani, 2011). The enzymes were dissolved completely before applying to the pigeon pea grains by smearing. For each experiment, 10 kg grains were passed through PKV mini dal mill for scarification (feed rate= 150 kg/h). After scarification (first pass) while passing through screw conveyor, the enzyme solution was applied by smearing (35-55 mg/100 g dm) to the scarified grains. The samples were incubated in hot air oven (Plate 8.4) at various incubation temperatures (35-55 ± 0.5 ^{o}C) and incubation periods (4-12 h). After incubation, the samples were dried in a tray dryer at 60 ^{o}C to reduce the moisture content to 10 ± 0.5 % (w.b.). Enzyme treated samples were spread in thin layer (25 mm) on a wire mesh tray for drying.

Enzyme treated samples of 10 kg weight having about 10 ± 0.5 % moisture content (w.b.) were milled using PKV mini dal mill. Samples were milled at the standard settings of the machine, i.e. 1420 rpm operating speed and 100 kg/h feed rate.

Design of experiments

The Box- Behnken design of three variables and three levels including 17 trials formed by 5 central points was used (Box and Behnken, 1960). This design was selected as it fulfils most of the requirements needed for optimization of dehulling of pigeon pea using enzyme treatment. The dehulling of pigeon pea assumed to be affected by three independent variables (regress or factors), *viz.,* enzyme concentration (X_1), incubation temperature (X_2) and incubation period (X_3). The experimental design of independent parameters and layout are shown in Tables 8.1 and 8.2 for these three levels and three variables under the Response Surface Methodology (Mullen and Ennis, 1979; Floros and Chinnan, 1987; Mudhar *et al.*, 1989; Pokharkar 1994; Saba 1994; Jain 2007; Ranmode, 2009, and Borkar, 2011). All these variables were closely controlled and accurately measured during experimentation.

Fixed parameters

1. Sample size, kg : 10
2. Enzyme proportion : 2:1:1 (xylanase : pectinase : cellulase)
3. pH : 5
4. Equipment : PKV mini dal mill

Table 1. Levels of independent variables for dehulling efficiency and dal recovery

Independent variables	Symbols		Levels	
	Coded	Un-coded	Coded	Un-coded
Enzyme concentration, mg/100 dm	x_1	X_1	1	55
			0	45
			-1	35
Incubation temperature, ^oC	x_2	X_2	1	55
			0	45
			-1	35
Incubation period, h	x_3	X_3	1	12
			0	8
			-1	4

Dependent variables

- Dehulling efficiency, %
- Dal Recovery, %

Design : Box Behnken

Analysis : Response Surface Methodology

Experimental procedure

The pigeon pea grains 10 kg of PKV Tara variety were cleaned graded and scarified/pitted by using PKV mini dal mil (I pass). The scarified grains were treated by smearing with enzyme solution of xylanase: pectinase: cellulase (2:1:1), the water of 5 pH was used for making enzyme solution in all treatments.

The concentration of enzyme solution, incubation temperature and incubation period were used as per Table 8.1. Then treated pigeon pea grains after drying to 10 % m.c. (w.b) were passed through PKV mini dal mil (2^{nd} pass). The dehulling efficiency and dal recovery was calculate by using equation 8.11 and 8.12 to avoid bias, 17 runs were performed in a random order for estimation of the constants of Eqn. 8.10. The decision for the range and centre points of the variables was taken through preliminary trials.

Numerical optimization

Numerical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. Each factor and response were chosen.

Table 2. Experimental layout for 3 variables and 3 levels response surface analysis

Tr. No.	Concentration of enzyme, mg/100 g d m	Incubation temperature, ^oC	Incubation period, h	Concentration of enzyme, mg/100 g d m	Incubation temperature, ^oC	Incubation period, h
	x_1	x_2	x_3	X_1	X_2	X_3
1	1	0	-1	55	45	4
2	1	0	1	55	45	12
3	0	0	0	45	45	8
4	0	0	0	45	45	8
5	-1	1	0	35	55	8
6	0	1	-1	45	55	4
7	1	-1	0	55	35	8
8	0	0	0	45	45	8
9	-1	0	-1	35	45	4
10	-1	-1	0	35	35	8
11	1	1	0	55	55	8
12	0	-1	1	45	35	12
13	0	0	0	45	45	8
14	-1	0	1	35	45	12
15	0	0	0	45	45	8
16	0	-1	-1	45	35	4
17	0	1	1	45	55	12

Dehulling data analysis

The data after milling was analysed using the following formulae:

Dehulling efficiency (DE)

Dehulling efficiency is an estimate of the efficiency of producing the major product. It was calculated using the following equation:

$$DE\ (\%) = 100\left(1-\frac{a_2}{a_1}\right)\times\frac{b_2-b_1}{(b_2-b_1)+(c_2-c_1)+(d_2-d_1)} \quad ...8.11$$

Where,

a_1 = % wt. of whole grain before dehulling

a_2 = % wt. of whole grain after dehulling

b_1 = % wt. of pearled grain plus splits before dehulling

b_2 = % wt. of pearled grain plus splits after dehulling

c_1 = % wt. of broken before dehulling

c_2 = % wt. of broken after dehulling

d_1 = % wt. of husk and powder before dehulling

d_2 = % wt. of husk and powder after dehulling

Dal recovery (DR)

Dal recovery was calculated using the following equation:

$$\text{Dal recovery (DR), \%} = (W_2 + W_4) \times 100/W_1 \quad \ldots 8.12$$

Where

W_1 = Initial weight of sample taken for dehulling, g

W_2 = Weight of dehulled grains, g

W_3= Weight of dehulled splits, g

The dehulling index and degree of dehulling at only optimized input parameters were analysed as shown in Table 8.10.

Dehulling index (DI)

The dehulling index was calculated using the following equation:

$$DI = \frac{(W_2 + W_h) - (W_3 - W_b)}{W_1} \quad ...8.13$$

Where,

W_1 = Initial weight of sample taken for dehulling, g

W_2 = Weight of dehulled grains, g

W_3 = Weight of undehulled grains, g

W_h = Weight of hulls, g

W_b = Weight of brokens and powder,

Degree of dehulling (DD)

The degree of hull removal is the percentage of dehulled kernels to the initial weight of sample taken for dehulling. The degree of dehulling will be defined using the following equation:

$$DD(\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad ...8.14$$

Cooking quality

Cooking time

The cooking time was determined by method used by Laxman Singh (1990), Sharma (1977), Shanta (1978).

Dispersed solids

Dispersed solids were assessed as the amount of dry matter released into the cooking water. The dry matter in the cooking water was determined gravimetrically by evaporating 25 ml of cooking water to dryness in air-oven at 105 ^{o}C. The residue was weighed and reported as a percentage of the original weight of the raw dried seeds

Results and discussion

Pigeon pea grains of variety PKV Tara were given different pretreatments before dehulling/milling in the present study. All the dehulling experiments were carried out at about 10 ± 0.5 % m.c. (w.b.).

Seed coat content

Seed coat content was calculated by the method as illustrated in material and methods. The PKV Tara grain contained 14.47 % seed coat on average basis.

Effect of Water Soaking Treatments

Minimum dehulling efficiency and dal recovery was observed in no soaking treatment to the tune of 62.78 % (± 0.9) and 51.23 % (± 0.71) respectively. The pretreatment of soaking in water for 5 min resulted in highest dehulling efficiency to the tune of 93.7 % (± 0.8) and highest dal recovery to the tune of 72.68 % (± 0.62) as compared to soaking in water for other durations (Table 4). The colour of final product was observed to be dull and slight deshaping in case of all soaking treatments. It was observed that as the soaking time increased, deshaping of final product was also found to be increased. Minimum deshaping was observed in water soaking treatment for 5 minutes. Broken, husk and powder per cent in case of 5 min soaking period was observed to be 2.4, 16.0 and 8.12 %, respectively.

Table 4. Dehulling of pigeon pea by water soaking treatment

Sr. No.	Treatment details	Dehulling efficiency, %	Dal recovery, %
1.	No Soaking	62.78 (± 0.9)	51.23 (± 0.71)
2.	Water soaking for 5 min.	93.7 (± 0.8)	72.68 (± 0.62)
3.	Water soaking for 10 min.	90.57 (± 0.8)	71.57 (± 0.5)
4.	Water soaking for 15 min.	85.07(± 0.85)	71.25 (± 0.68)
5.	Water soaking for 20 min.	85.7 (± 0.85)	70.7 (± 0.81)
6.	Water soaking for 20 min.	69.6 (±0.8)	70.56 (±0.83)

* Figures in paranthesis represent standard deviation

Effect of Oil Treatment (Dry Method)

The results regarding dehulling efficiency and dal recovery by oil treatment are shown in Table 5.

Table 5. Dehulling of pigeon pea by oil treatment

Sr. No.	Treatment details	Dehulling efficiency, %	Dal recovery, %
1	Oil treatment	93.8 (±0.92)	74.79 (±0.75)

For oil treatment dehulling efficiency and dal recovery was found to be 93.8 % and 74.79 %, respectively. The per cent husk removed in case of oil treatment was higher than that of water treatment and also deshaping was not observed as in case of water soaking treatment and hence the quality was found better compared to soaking in water for 5 min. Broken, husk and powder per cent was observed to be 2.0, 15.0 and 8.0 %, respectively.

Optimization of Dehulling of Pigeonpea by Enzymic Treatment

The optimization of process parameters such as enzyme concentration, incubation temperature and incubation period is necessary so that maximum dehulling efficiency and dal recovery could be achieved. As per 3 variable 3 level Box Behnken model, 17 trials were performed as enumerated in Table 8.6 for obtaining the dehulling efficiency and dal recovery responses for each treatment. All these trials were conducted with 10 kg sample size and data for dehulling efficiency and recovery was reported. The process of dehulling of pigeon pea by enzymatic treatment is given in flowchart (Fig. 8.3). To avoid bias, 17 runs were performed in a random order for estimation of constant of equation 8.1. The decision for the range and centre points of the variables was taken through preliminary trials. The independent variables i.e. enzyme concentration, incubation temperature and incubation period, the coded variables (X_1), decoded variables and their levels are presented Table 8.4, as described by Pokharkar (1994), Chowdhury *et al.* (2000), Ravindra and Chattopadhyay (2000), Jain (2007), Singh *et al.* (2008), Ranmode (2009) and Borkar (2011).

The dehulling efficiency was in the range of 91.00 to 96.60 per cent and dal recovery was in the range of 69.5 to 77.05 per cent.

The degumming may be due to the action of different enzymes used for treatment, i.e., xylanase, pectinase and cellulase. The combination of these three enzymes at different proportion reacts on the gums which is basically a polymer of sugar and acid. By the action of enzymes, the polymeric compound degrades to monomeric units resulting in loosening of the husk.

8.4.1 Effect of enzymatic treatments on dehulling efficiency

The results regarding enzymatic treatments are given in Table 8.7. The dehulling efficiency was observed to be ranging from 91.00 to 96.6 % depending upon the enzyme treatments. The minimum dehullig efficiency was found for treatment having the combination of enzyme concentration of 35 mg/100 g dry matter, 55^{o}C incubation temperature and 8 h incubation period. The maximum dehulling efficiency was observed in case of treatment having the combination of enzyme concentration 45 mg/100 g dry matter, incubation temperature 45 ^{o}C and incubation period 8 h.

Table 8.7. Effect of various levels of enzymatic treatment on dehulling efficiency and dal recovery

Tr. No.	Enzyme concentration (mg/100 g dm)	Incubation temperature (^{o}C)	Incubation period (h)	Dehulling Efficiency (%)	Dal recovery (%)
1.	55	45	4	94.9	72.6
2.	55	45	12	94.6	71.8
3.	45	45	8	96.6	77.04
4.	45	45	8	94.5	75.58
5.	35	55	8	91	70.55
6.	45	55	4	92.8	74
7.	55	35	8	93.5	69.15
8.	45	45	8	96.6	77.05
9.	35	45	4	93	73.8
10.	35	35	8	92.5	69.8
11.	55	55	8	92.8	69.92
12.	45	35	12	93.2	69.2
13.	45	45	8	96.52	76.46
14.	35	45	12	93	73.5
15.	45	45	8	96.6	76.8
16.	45	35	4	93.6	69.5
17.	45	55	12	92.4	73.5

The analysis of variance (ANOVA) was made for the experimental data and the significance of enzyme concentration, incubation temperature and incubation period as well as their interactions on dehulling efficiency was analyzed. The response surface quadratic model was

fitted to the experimental data and statistical significance of linear, interaction and quadratic effects were analyzed for dehulling efficiency response (Table 8.8). It revealed that the model was highly significant at 1 % level of significance.

The results showed that among linear effects, enzyme concentration had significant effect on dehulling efficiency ($P<0.05$) at 5 % level of significance followed by incubation temperature and the effect of incubation period was found not significant. Quadratic effect of enzyme concentration, incubation temperature and incubation period had significant effect on dehulling efficiency ($P<0.05$) at 5 % level of significance. The existence of quadratic terms indicates the curvy linear nature. It indicates that increasing the value of variable initially increases the response up to certain level of variable however further increase in the level of variable decreases the value of response.

The dehulling efficiency varied from 91.0 to 96.6 % (Table 8.7). The data regarding weight of different fractions broken, husk and powder, dal recovery and dehulling efficiency obtained with different combination of variables are given in Appendix E. The minimum dehulling efficiency was found in treatment having the combination of enzyme concentration of 35 mg/100 g dry matter, 55 ^{o}C incubation temperatures and 8 h incubation period while the maximum dehulling efficiency found in treatment having the combination of enzyme concentration of 45 mg/100 g dry matter, 45 ^{o}C incubation temperatures and 8 h incubation period. The quadratic response surface model data indicated the results as significant. The lack of fit was found to be non significant and hence the model was significant. The coefficient of determination (R^2) was 0.9208 for enzymatic treatment which indicated that the model could fit the data for enzyme activity very well for all the three variables, i.e. enzyme concentration, incubation temperature and incubation period. The response surface equation was obtained for the model of second degree in terms of coded factors as under.

$$\text{Dehulling efficiency},\% = 96.16+0.79X_1 - 0.47X_2 - 0.14X_3 - 1.42X_1^2 - 2.29X_2^2 - 0.87X_3^2 \quad ...8.15$$

Where,

X_1 = enzyme concentration (mg/100 g dm)

X_2 = incubation temperature, ^{o}C

X_3 = incubation period, h

Table 8.8. ANOVA for effect of enzymatic treatment variables on dehulling efficiency

Source	Df	Sum of Square	Mean sum of square	F Value	p-value Prob>Fig	
Model	**6**	**44.00**	**7.33**	**19.37**	**0.0001**	**Significant**
X_1 : Enzyme concentration	1	4.96	4.96	13.10	0.0047	
X_2 : Incubation temperature	1	1.80	1.80	4.77	0.0539	
X_3 : Incubation period	1	0.15	0.15	0.40	0.5415	
X_1^2	1	8.48	8.48	22.41	0.0008	
X_2^2	1	22.17	22.17	58.55	0.0001	
X_3^2	1	3.18	3.18	8.41	0.0158	
Residual	10	3.79	0.38			
Lack of Fit	6	0.32	0.053	0.062	0.9977	NS
Pure Error	4	3.47	0.87			
Correlation Total	16	47.79				

NS – Non significant

8.4.1.1 Effect of enzyme concentration and incubation temperature on dehulling efficiency

The effect of enzyme concentration and incubation temperature on dehulling efficiency was determined keeping incubation period constant at 8 h which is shown in Fig. 8.4. Three dimensional responses for dehulling efficiency of enzyme treated samples were generated. From these surfaces, it could be evident that dehulling efficiency initially increased with increase in enzyme concentration and incubation temperature and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Enzyme concentration and incubation temperature had shown significant effect on dehulling efficiency. It was observed that with increase in enzyme concentration and incubation temperature, the dehulling efficiency increased at a particular enzyme concentration and incubation temperature. The reduction in enzymatic activity at above optimum incubation temperature was due to denaturing of enzyme, resulting in the reduction in dehulling efficiency. It also confirmed the facts that maximum enzymatic reaction occurred at optimum enzyme concentration and temperature levels.

8.4.1.2 Effect of enzyme concentration and incubation period on dehulling efficiency

The effect of enzyme concentration and incubation period on dehulling efficiency was determined keeping incubation temperature constant at 45 ^{o}C (Fig. 8.5). It could be observed that with increase in incubation period, the dehulling efficiency increased at a particular enzyme concentration. It also confirms the findings that the dehulling efficiency first increased with enzyme concentration and incubation period and then decreased. The

reduction in activity at higher enzyme concentration above certain optimum level might be due to saturation of active sites of enzymes with substrate leading to lower dehulling efficiency. However, the effect of enzyme concentration on dehulling efficiency was found to be highly significant. Higher incubation period might have produced inhibitor substances for enzyme action resulting in lower dehulling efficiency. The effect of incubation period was found to be non significant at 5 % level of significance.

8.4.1.3 Effect of incubation period and incubation temperature on dehulling efficiency

The effect of incubation period and incubation temperature on dehulling efficiency is shown in Fig. 8.6. Three dimensional responses for dehulling efficiency of enzyme treated samples were generated. From these surfaces, it could be evident that dehulling efficiency initially increased with increase in incubation period and incubation temperature and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Incubation temperature showed significant effect on dehulling efficiency. However, it was observed that the dehulling efficiency increased with increase in incubation period was small as compared to incubation temperature. It was also observed that with increase in incubation temperature, the dehulling efficiency increased at a particular incubation period. The reduction in enzyme activity above certain optimum incubation temperature would denature the enzymes, resulting in decrease in the dehulling efficiency.

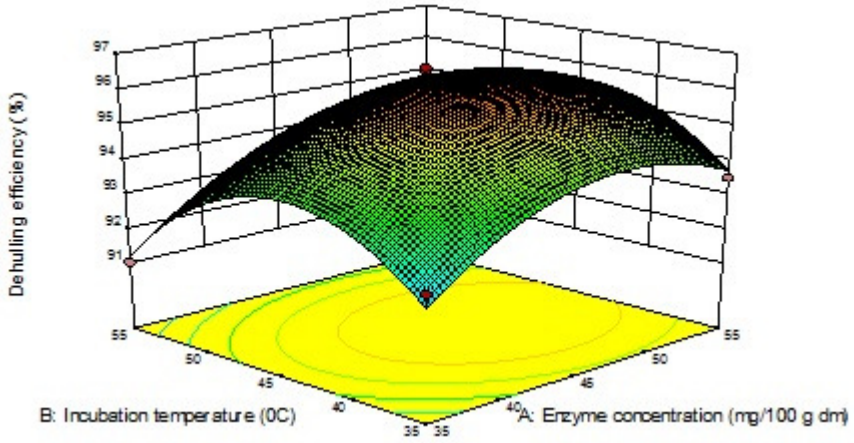


Fig. 8.4. Effect of enzyme concentration and temperature on dehulling efficiency

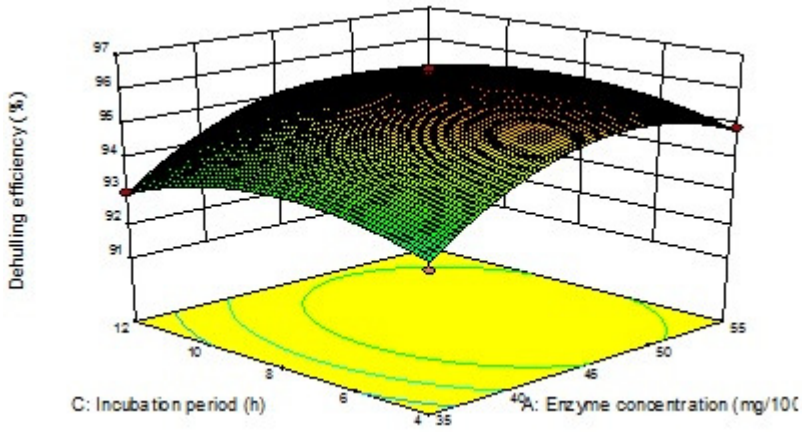


Fig 8.5. Effect of enzyme concentration and incubation period on dehulling efficiency

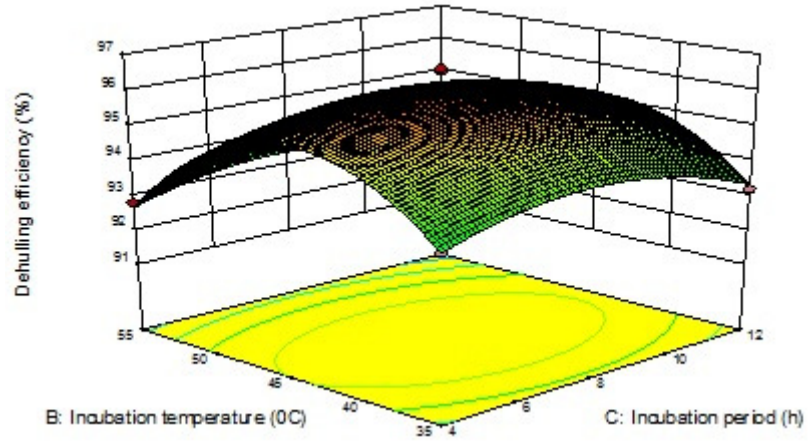


Fig 8.6. Effect of incubation period and incubation temperature on dehulling efficiency

8.4.2 Effect of enzyme treatments on dal recovery

The results regarding dal recovery by using various enzymatic treatments are given in Table 8.7.

From Table 8.7, it revealed that the dal recovery was observed to be ranging from 69.15 to 77.05 % depending upon the enzymatic treatments. The minimum dal recovery was found for treatment having the combination of enzyme concentration of 55 mg/ 100 g dry matter, incubation temperature 35 ^{o}C and incubation period 8 h. The maximum dal recovery was observed in case of treatment having the combination of enzyme concentration 45 mg/ 100 g dry matter, incubation temperature 45 ^{o}C and incubation period 8 h.

The ANOVA revealed that the model was highly significant at 5 % level of significance. The results showed that among linear effects, incubation period had significant effect on dal recovery ($P<0.05$) at 5 % level of significance. However, linear effects of enzyme concentration and incubation period were found to be non significant. The interaction terms were also found absent indicating non-significant effect. Incubation temperature and enzyme

concentration had significant effect on dal recovery (P<0.05) at 5 % level of significance. The existence of quadratic terms indicates curvy linear nature. It indicates that increasing the value of variable initially increases the response upto certain level of variable however further increase in the level of variable decreases the value of response.

The dal recovery varied from 69.15 to 77.05 % (Table 8.7). The data regarding weight of different fractions i.e. broken husk and powder and response i.e. dal recovery obtained with different combination of variables are given in Appendix E. The minimum dal recovery was found in treatment having the combination of 55 mg/ 100 g dry matter enzyme concentration, 35 ^{o}C incubation temperature and 8 h incubation period while the maximum dal recovery found in treatment having the combination of 45 mg/ 100 g dry matter enzyme concentration, 45 ^{o}C incubation temperature and 8 h incubation period. The quadratic response surface model data indicated the results as significant. The lack of fit was non significant and hence the model was significant. The coefficient of determination (R^2) was 0.9376 for enzymatic pre-treatment which indicated that the model could fit the data for enzyme activity very well for all the three variables, i.e. enzyme concentration, incubation temperature and incubation period.

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

$$\text{Dal recovery, \%} = 76.59 - 0.52\ X_1 + 1.29X_2 - 0.24\ X_3 - 2.68X_1^2 - 4.05X_2^2 - 0.98X_3^2 \quad \ldots 8.16$$

Where,

X_1 = enzyme concentration, mg/ 100 g dm

X_2 = incubation temperature, ^{o}C

X_3 = incubation period, h

Table 8.10. ANOVA for effect of enzyme treatment variables on dal recovery

Source	Df	Sum of Square	Mean sum of square	F Value	p-value Prob>F
Model	**6**	**128.21**	**21.37**	**25.05**	**0.0001(S)**
X_1 : Enzyme concentration	1	2.18	2.18	2.56	0.1407
X_2 : Incubation temperature	1	13.31	13.31	15.7	0.0027
X_3 : Incubation period	1	0.45	0.45	0.53	0.4837
X_1^2	1	30.20	30.20	35.29	0.0001
X_2^2	1	69.17	69.17	81.07	0.0001
X_3^2	1	4.07	69.17	4.77	0.0539

Residual	1	8.13	4.07		
Lack of Fit	10	7.04	0.85	3.14	0.144(NS)
Pure error	4	1.50	1.17		
Correlation Total	16	136.74	0.37		

NS= Non significant S= Significant

8.4.2.1 Effect of enzyme concentration and incubation temperature on dal recovery

The effect of enzyme concentration and incubation temperature on dal recovery was determined keeping incubation period constant at 8 h which is shown in Fig. 8.7. Three dimensional responses for dal recovery of enzyme treated samples were generated. From these surfaces, it could be evident that dal recovery initially increased with increase in incubation temperature and enzyme concentration and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Incubation temperature had shown significant effect on dal recovery. It was observed that with increase in incubation temperature, the dal recovery increased at a particular enzyme concentration. The reduction in enzymatic activity above certain optimum temperature was due to denaturing of enzyme, resulting in the reduction in dal recovery. It also confirmed the fact that maximum enzymatic reaction occurred at optimum temperature levels.

8.4.2.2 Effect of enzyme concentration and incubation period on dal recovery

The effect of enzyme concentration and incubation period on dal recovery was determined keeping incubation temperature constant at 45 ^{o}C (Fig. 8.8). It could be observed that with increase in incubation period, the dal recovery increased at a particular enzyme concentration. It also confirms the findings that dal recovery first increases with incubation period and enzyme concentration and then decreases. The reduction in activity at higher enzyme concentration might be due to saturation of active sites of enzymes with substrate leading to lower dal recovery. However, the effect of enzyme concentration on dal recovery was found to be non significant. Higher incubation period might have produced inhibitor substances for enzyme action resulting in lower dal recovery.

The minimum dal recovery of 69.15 % was obtained for the combination of 55 m g/ 100 g dry matter enzyme concentration, 35 ^{o}C incubation temperature and 12 h incubation period whereas the maximum dal recovery of 77.05 % was found for combination of 45 mg/ 100 g dry matter enzyme concentration, 45 ^{o}C incubation temperature and 8 h incubation period. This showed that incubation temperature was more effective than the enzyme concentration and incubation period on dal recovery.

8.4.2.3 Effect of incubation period and incubation temperature on dal recovery

The effect of incubation period and incubation temperature on dal recovery is shown in Fig. 8.9. Three dimensional responses for dal recovery of enzyme treated samples were generated. From these surfaces, it could be evident that dal recovery initially increased with increase in incubation period and incubation temperature and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Incubation temperature was showing significant effect on dal recovery. However, it was observed that the dal recovery increased with increase in incubation period was small as compared to incubation temperature. It was also observed that with increase in incubation temperature, the dal recovery increased at a particular incubation period. The reduction in enzyme activity above certain optimum incubation temperature would denature the enzymes, resulting in decrease in the dal recovery.

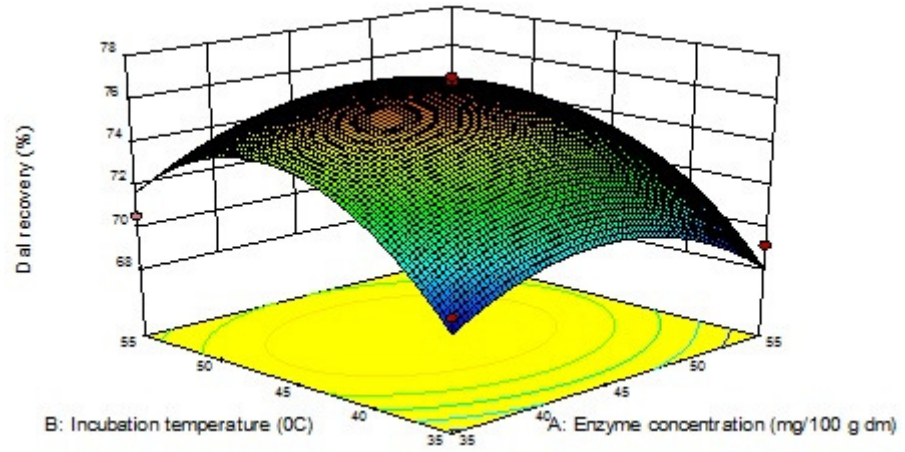


Fig. 8.7. Effect of enzyme concentration and incubation temperature on \ dal recovery

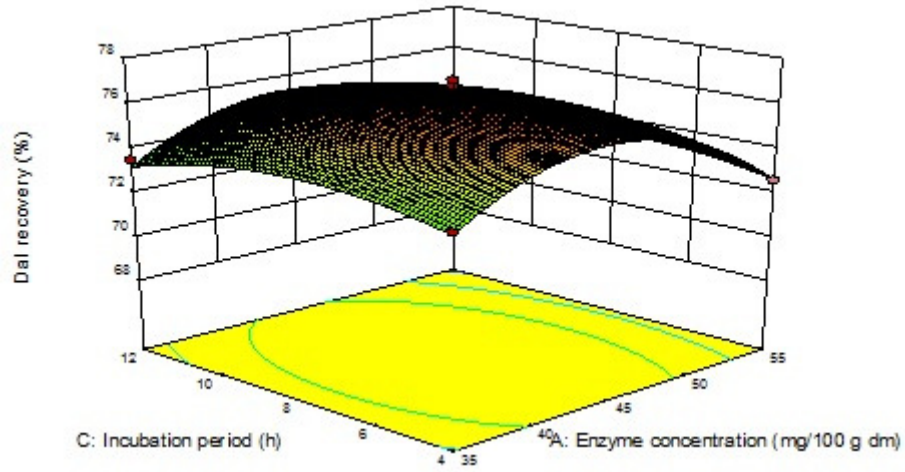


Fig. 8.8. Effect of enzyme concentration and incubation period on dal recovery

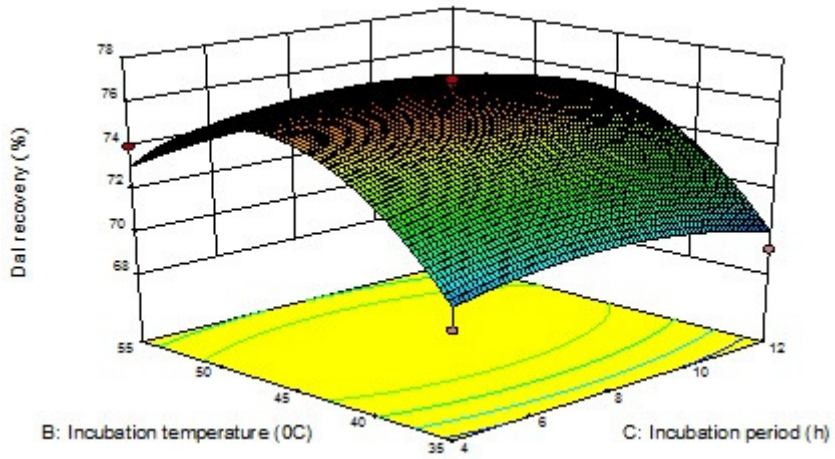


Fig. 8.9. Effect of period and incubation temperature on dal recovery

8.4.3 Optimization of enzyme treatment variables

Software Design Expert version 9.0.3.1 was used for the optimization of responses. A stationary point at which the slope of the response surface was zero in all the direction 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 values of enzymatic hydrolysis pretreatment were evaluated using equation 8.1 and 8.2 the multiple regression package was used for purpose. The response surface quadratic model optimized pretreatment as enzyme concentration 45.64 mg /100 g dm, incubation temperature, 45.42 ^{o}C, Incubation period 7.61 h, which gave predicted value of dehulling efficiency 96.19 % and dal recovery 76.6 %. The optimum values for different variables and their predicted responses thus obtained are given in Table 8.11 as well as Fig. 8.10 and 8.11.

Table 8.11. Optimized variables and their responses for enzyme pre-treatment of pigeon pea grains

Variable	Optimized values	Responses	Predicted values
Enzyme concentration, mg/100 g dm	45.64	Dehulling efficiency %	96.19
Incubation period, h	7.61	Dal recovery %	76.60
Incubation temperature, ^{o}C	45.42		
R^2	0.935		

The optimum values of different variables for enzymatic treatment were found within the range considered in the study.

8.4.4 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 treatment variables, the experiment was conducted at derived conditions. The data regarding dehulling efficiency and

dal recovery obtained of optimized condition of variable is given in Table 8.12. From the Table 8.12, it could be seen that the predicted values of dehulling efficiency and dal recovery were 96.19 % and 76.16 % respectively. These were experimentally verified in the laboratory and observed values of dehulling efficiency and dal recovery were found to be 96.05 % and 76.84 % respectively. It could reveal that the experimental values were very close to the predicted values which confirmed the optimum conditions.

Table 8.12. Predicted and experimental values of responses at optimum level of different variables

Sr. No.	Responses	Predicted values	Experimental values
1	Dehulling efficiency, %	96.19	96.05 (± 1.26)
2	Dal recovery, %	76.16	76.84 (± 1.05)
3	Dehulling index	-	0.056 (± 0.15)
4	Degree of dehulling, %	-	92.65 (± 1.71)

* Figures in paranthesis represent standard deviation

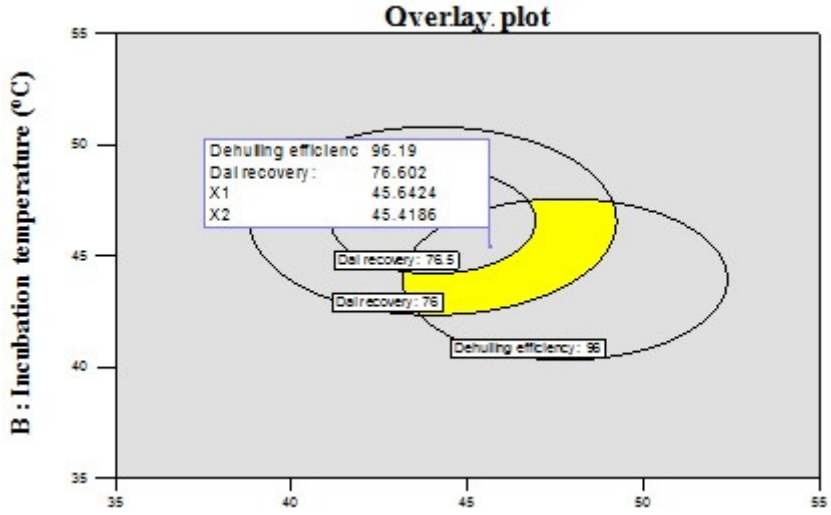


Fig. 8.10. Effect of enzyme concentration and incubation temperature on dehulling efficiency and dal recovery

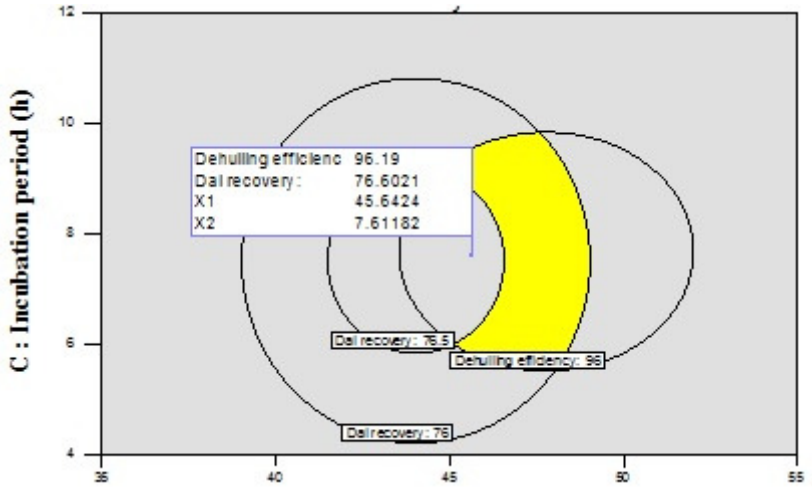


Fig. 8.11. Effect of enzyme concentration and incubation period on dehulling efficiency and dal recovery

8.5 Nutritional and Cooking Quality

8.5.2 Cooking time

The results of the cooking studies (cooking in boiling water) of dal, i.e., the finished product obtained under different standardized pretreatments are given in Table 8.14.

Table 8.14. Cooking time of dal obtained under different pretreatments

Sr. No.	Pretreatments	Cooking time (min)			
		I	II	Av.	Time saving Over
1	Optimized water soaking treatment (5 min)	26	26	26.0	0.0
2	Optimized enzyme treatment	23	23	23.0	3.0
3	Oil treatment	24	24	24	2.0

It could be seen from the Table 8.14 that water soaking treatment required highest cooking time of 26 min. Whereas the enzyme treated and dehulled samples required the cooking time of 23.0 min. and oil treated and dehulled sample required 24 min for cooking. Enzyme treated and dehulled samples required 3 min and 2 min less time for cooking compared to water soaked and dehulled sample i.e. Enzyme treated samples required 7.70 % less time for cooking than water soaked samples. It revealed that no significant change in cooking time was observed for water soaked, oil and enzyme treated samples.

8.5.3 Dispersed solids

Dispersed solids were assessed as given in section 8.6.3. Dispersed solids in water soaked, oil treated and enzyme treated dehulled samples were found to be 10.04 ± 0.3, 10.4 ± 0.5, and 9.4 ± 0.8 %, respectively. The result revealed that the enzyme treatment and water soaking did not alter the solid dispersion compared to oil treatment

The enzymatic treatment gave about 96.19 % and 77.05 % higher dehulling efficiency and dal recovery as compared to traditionally practiced water soaking treatment. The cooking time was reduced by 3 min. while the protein content of pigeon pea dal increased by 2.96 % compared to water soaking treatment of pigeon pea milling. The quantity of enzymes required had been estimated considering the 10 % (w.b.) moisture content of the pigeon pea grains.

Table 8.15. Comparison of cost and quality of different dehulling Methods

Treatment / Particulars	Dehulling by oil treatment (dry method)	Dehulling by water soaking treatment	Dehulling by enzymatic treatment
Cost Rs./q	230	100	145
Quality	Grade I dal	Grade II dal	Better than Grade II near to grade I

From Table 8.15, It revealed that the cost of operation of pigeon pea dehulling by water soaking method was observed to be Rs. 100/q which is much less than conventional / commercial method of oil treatment whereas the cost of operation by enzymatic treatment was Rs. 145 per quintal which is nearer to water soaking treatment. Regarding quality, the dal obtained by water soaking treatment was of grade II quality (slightly deshaped), however the dal obtained by conventional/ commercial method (oil treatment) was of good quality i.e. grade I dal whereas the dal obtained by the enzymatic treatment gave about 96.19 % and 77.05 % higher dehulling efficiency and dal recovery as compared to traditionally practiced oil milling treatment. The cooking time was reduced by 3 min. while the protein content of pigeon pea dal increased by 2.96 % by following suggested enzymatic pretreatment of pigeon pea milling. The quantity of enzymes required had been estimated considering the 10 % (w.b.) moisture content of the pigeon pea grains.

Conclusion

Based on the results reported, it could be concluded that the enzyme solution having 2:1:1 proportion of xylanase, pectinase and cellulase enzymes should be prepared and applied at the rate of 45 mg/100 g dm of dry pigeon pea grain. The enzyme treated pigeon pea grains should be kept at 45.64 ^{o}C incubation temperature for 7.6 h incubation period. The observed values of dehulling efficiency, dal recovery, protein content and cooking time at the suggested conditions of enzyme treatment variables were 96.19 %, 76.60 %, 18.56 % and 23 min, respectively.

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