ENZYMATIC PRE-TREATMENTS ON PIGEON PEA FOR BETTER RECOVERY AND QUALITY OF DHAL

A THESIS SUBMITTED TO THE JUNAGADH AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN AGRICULTURAL ENGINEERING (AGRICULTURAL PROCESS AND FOOD ENGINEERING)

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Pigeon pea (Cajanus cajan L.) is one of the important pulse crops of India contributing 20.87% to the total production of all pulses. India accounts for 90% of the total world production of pigeon pea. Pigeon pea is significantly contributing to meet the dietary requirement of crude fibre, ash, fat, magnesium, manganese and copper. Pigeon pea contains high amount of vitamin B, Carotene and ascorbic acid. Pigeon pea is mainly consumed as dhal because it takes less time to cook and has acceptable appearance, texture, palatability, digestibility, and overall nutritional quality.

The pigeon pea grain is considered as most difficult for dehulling as compared to other pulses owing to its seed coat which is more firmly attached with the cotyledons through a layer of gum and mucilage. The primary objective of dehulling is to remove seed coat from the cotyledons, during which four different fractions, i.e., dhal, broken, powder and husk are obtained. Pre-milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion.

The experiments mainly consisted of physicochemical properties of pigeon pea grains, scanning electron microscopy of enzyme treated grains, enzymatic pre-treatments on milling quality, protein content and cooking time. Different properties of pigeon pea grains, namely size in terms of length, breath and thickness, sphericity, bulk density, porosity, true density, angle of repose and coefficient of static friction against different surfaces were determined at 10, 15, 20, 25 and 30% (d.b.) moisture.
content. The proximate compositions of pigeon pea grains, viz., carbohydrate, protein, fat, crude fibre and ash were determined at 10.46 %, (w.b.) moisture content.

The effect of four enzymatic hydrolysis parameters viz., enzyme concentration (20, 30, 40, 50 and 60 mg/100 g dry matter), incubation time (3, 6, 9, 12 and 15 h), incubation temperature (40, 45, 50, 55 and 60 °C) and tempering water pH (4.0, 4.5, 5.0, 5.5 and 6.0) on hulling efficiency, protein content and cooking time were optimized using response surface methodology. For the comparison of enzymatic pre-treatment, the dry milling method was considered as control.

Microstructure of all the enzymatically hydrolyzed as well as oil treated (control) samples were examined using a Scanning Electron Microscope. Sensory evaluation of the cooked samples of enzyme treated and control samples was carried out immediately after cooking in terms of colour, appearance, flavour, texture, taste and overall acceptability.

The average length, width, thickness, size and thousand grain mass of pigeon pea grains increased from 6.05 to 6.32 mm, 5.43 to 5.63 mm, 4.64 to 4.71 mm, 5.337 to 5.510 mm and 97.90 to 116.83 g with the increase in moisture content from 10 to 30 % (d.b.). The sphericity, bulk density and true density decreased logarithmically from 0.883 to 0.871, 872 to 814 kg/m³ and 1353 to 1307 kg/m³ with the increase in moisture content from 10 to 30 % (d.b.), respectively. The porosity and angle of repose of pigeon pea grains increased logarithmically from 35.47 to 37.96 % and 28.17° to 34.08° with increasing moisture content from 10 to 30 % (d.b.), respectively. At all the moisture contents, the static coefficient of friction was highest against plywood surface which ranged from 0.41 to 0.62, for galvanized sheet from 0.34 to 0.52 and lowest for glass surface that is from 0.336 to 0.456. The moisture content of pigeon pea grains was found to be 10.46 % (w.b.), protein 18.73 %, carbohydrate 58.15 %, fat 1.62 %, crude fibre 7.45 %, total ash 3.70 %.

The cavity thickness observed through sectional images of enzyme treated pigeon pea samples using scanning electron microscope varied from 3.80 to 48.84 µm. It was observed that the cavity thickness of enzymatic treated samples increased which resulted in to the increase in the percentage husk removed.
From the above study, it could be recommended that the better recovery and quality of pigeon pea dhal could be obtained by enzymatic pre-treatment of enzyme concentration of 37.80 mg/100 g dry matter, 8.69 h incubation time, 48.5 °C incubation temperature and 5.49 tempering water pH for obtaining a hulling efficiency 88.12 % with 21.81 % protein content and 21.5 min cooking time for dhal. This could increase hulling efficiency by 13.47 %, protein content by 12.33 % and decrease in cooking time by 19.77 % as compared to the control. The sensory evaluation indicated that the dhal obtained through enzymatic pre-treatment had higher value of overall acceptability as compared to control sample.

Key words: pigeon pea, enzymatic pre-treatment, hulling efficiency, protein content, cooking time, scanning electron microscopy
This is to certify that the thesis entitled “ENZYMATIC PRE-TREATMENTS ON PIGEON PEA FOR BETTER RECOVERY AND QUALITY OF DHAL”, submitted by Mr. SANGANI VELJI PREMJI (Registration No. J4-000158-2005) in partial fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY in AGRICULTURAL ENGINEERING with specialization in AGRICULTURAL PROCESS AND FOOD ENGINEERING of the JUNAGADH AGRICULTURAL UNIVERSITY is a record of bonafide research work carried out by him under my guidance and supervision; and the thesis has not been previously formed the basis for the award of any degree, diploma or other similar title.

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<td>Association of Official Analytical Chemists</td>
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<td>c.c.</td>
<td>cubic centimetre</td>
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<tr>
<td>CD</td>
<td>critical difference</td>
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<tr>
<td>CCD</td>
<td>Central Composite Design</td>
</tr>
<tr>
<td>CCRD</td>
<td>Central Composite Rotatable Design</td>
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<tr>
<td>CFTRI</td>
<td>Central Food Technological Research Institute</td>
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<tr>
<td>CIRCOT</td>
<td>Central Institute for Research on Cotton Technology</td>
</tr>
<tr>
<td>$C_h$</td>
<td>Coefficient of hulling</td>
</tr>
<tr>
<td>$C_{wk}$</td>
<td>Coefficient of wholeness of kernel</td>
</tr>
<tr>
<td>CIAE</td>
<td>Central Institute of Agricultural Engineering</td>
</tr>
<tr>
<td>CLEA</td>
<td>Cross-linked enzyme aggregates</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>CV</td>
<td>Co-efficient of Variance</td>
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<td>d.b.</td>
<td>dry basis</td>
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<td>D.F</td>
<td>degree of freedom</td>
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<td>DM</td>
<td>dry matter</td>
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<td>e.g.</td>
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etc.  et cetera
FAO  Food and Agricultural Organization
FMRC  Farm Machinery Research Centre
Fig.  Figure
g  gram
h  hour
HCl  Hydrochloric acid
hp  Horse Power
H₂SO₄  Sulphuric acid
i.e.  id est (that is)
ICAR  Indian Council of Agricultural Research
IIPR  Indian Institute of Pulse Research
Inst.  Institute
Int.  International
J  Journal
K  Potash
₀K  Degree Kelvin
kg  kilogram
kgf  kilogram force
kcal  Kilo calorie
L  litre
LSU  Louisiana State University
S.Em  Standard error of mean
m  meter
m.c.  moisture content
Mg  Magnesium
µg  microgram
µmol  micromole
min  minute
mg  milligram
mL  milliliter
mm  millimeter
Na  Sodium
NaHCO₃  Sodium bicarbonate
NIR  Near Infra Red
NSP  Non Starch Polysaccharides
P  pressure
p  page
pH  Negative logarithm of the effective hydrogen ion concentration
Pvt.  Private
Res.  Research
rpm  revolution per minute
RSM  Response Surface Modelling
s  second
Soc.  Society
U  Enzyme unit
UV  Ultra Violet
w.b.  wet basis
β  beta
µ  micron
µm  micrometer
%  per cent
>  Greater than
Viz.  Namely
XYU  Units of Xylanase
Pigeon pea (Cajanus cajan L.) is one of the important pulse crops of India contributing 20.87% to the total production of all pulses. India accounts for 90% of the total world production of pigeon pea. Pigeon pea is significantly contributing to meet the dietary requirement of crude fibre, ash, fat, magnesium, manganese and copper. Pigeon pea contains high amount of vitamin B, Carotene and ascorbic acid. Pigeon pea is mainly consumed as dhal because it takes less time to cook and has acceptable appearance, texture, palatability, digestibility, and overall nutritional quality.

The pigeon pea grain is considered as most difficult for dehulling as compared to other pulses owing to its seed coat which is more firmly attached with the cotyledons through a layer of gum and mucilage. The primary objective of dehulling is to remove seed coat from the cotyledons, during which four different fractions, i.e., dhal, broken, powder and husk are obtained. Pre milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion.

The experiments mainly consisted of physicochemical properties of pigeon pea grains, scanning electron microscopy of enzyme treated grains, enzymatic pre-treatments on milling quality, protein content and cooking time. Different properties of pigeon pea grains, namely size in terms of length, breath and thickness, sphericity, bulk density, porosity, true density, angle of repose and coefficient of static friction against different surfaces.
were determined at 10, 15, 20, 25 and 30 % (d.b.) moisture content. The proximate compositions of pigeon pea grains, viz., carbohydrate, protein, fat, crude fibre and ash were determined at 10.46 %, (w.b.) moisture content.

The effect of four enzymatic hydrolysis parameters viz., enzyme concentration (20, 30, 40, 50 and 60 mg/100 g dry matter), incubation time (3, 6, 9, 12 and 15 h), incubation temperature (40, 45, 50, 55 and 60 °C) and tempering water pH (4.0, 4.5, 5.0, 5.5 and 6.0) on hulling efficiency, protein content and cooking time were optimized using response surface methodology. For the comparison of enzymatic pre-treatment, the dry milling method was considered as control.

Microstructure of all the enzymatically hydrolyzed as well as oil treated (control) samples were examined using a Scanning Electron Microscope. Sensory evaluation of the cooked samples of enzyme treated and control samples was carried out immediately after cooking in terms of colour, appearance, flavour, texture, taste and overall acceptability.

The average length, width, thickness, size and thousand grain mass of pigeon pea grains increased from 6.05 to 6.32 mm, 5.43 to 5.63 mm, 4.64 to 4.71 mm, 5.337 to 5.510 mm and 97.90 to 116.83 g with the increase in moisture content from 10 to 30 % (d.b.). The sphericity, bulk density and true density decreased logarithmically from 0.883 to 0.871, 872 to 814 kg/m$^3$ and 1353 to 1307 kg/m$^3$ with the increase in moisture content from 10 to 30 % (d.b.), respectively. The porosity and angle of repose of pigeon pea grains increased logarithmically from 35.47 to 37.96 % and 28.17° to 34.08° with increasing moisture content from 10 to 30 % (d.b.), respectively. At all the moisture contents, the static coefficient of friction was highest against plywood surface which ranged from 0.41 to 0.62, for galvanized sheet from 0.34 to 0.52 and lowest for glass surface that is from 0.336 to 0.456. The moisture content of pigeon pea grains was found to be 10.46 % (w.b.), protein 18.73 %, carbohydrate 58.15 %, fat 1.62 %, crude fibre 7.45 %, total ash 3.70 %.

The cavity thickness observed through sectional images of enzyme treated pigeon pea samples using scanning electron microscope varied from
3.80 to 48.84 µm. It was observed that the cavity thickness of enzymatic treated samples increased which resulted in to the increase in the percentage husk removed.

From the above study, it could be recommended that the better recovery and quality of pigeon pea dhal could be obtained by enzymatic pre-treatment of enzyme concentration of 37.80 mg/100 g dry matter, 8.69 h incubation time, 48.5 °C incubation temperature and 5.49 tempering water pH for obtaining a hulling efficiency 88.12 % with 21.81 % protein content and 21.5 min cooking time for dhal. This could increase hulling efficiency by 13.47 %, protein content by 12.33 % and decrease in cooking time by 19.77 % as compared to the control. The sensory evaluation indicated that the dhal obtained through enzymatic pre-treatment had higher value of overall acceptability as compared to control sample.

Key words: pigeon pea, enzymatic pre-treatment, hulling efficiency, protein content, cooking time, scanning electron microscopy
This is to certify that the thesis entitled “ENZYMATIC PRE-TREATMENTS ON PIGEON PEA FOR BETTER RECOVERY AND QUALITY OF DHAL”, submitted by Mr. SANGANI VELJI PREMJI (Registration No. J4-000158-2005) in partial fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY in AGRICULTURAL ENGINEERING with specialization in AGRICULTURAL PROCESS AND FOOD ENGINEERING of the JUNAGADH AGRICULTURAL UNIVERSITY is a record of bonafide research work carried out by him under my guidance and supervision; and the thesis has not been previously formed the basis for the award of any degree, diploma or other similar title.

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Date : 30-11-2012

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Vice Chancellor
Junagadh Agricultural University
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This is to certify that the thesis entitled “ENZYMATIC PRE-TREATMENTS ON PIGEON PEA FOR BETTER RECOVERY AND QUALITY OF DHAL” submitted for the degree of Ph. D. (Agril. Engg.) in the subject of AGRICULTURAL PROCESS AND FOOD ENGINEERING embodies bonafide research work carried-out by Mr. SANGANI VELJI PREMJI under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

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Date :  30-11-2012                                                                    V P. Sangani
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5.3 Dhal obtained from enzymatic and oil pre treatment samples

5.4 Cooked dhal and blended samples obtained from oil and enzymatic pre treatment
NOMENCLATURE

ANOVA Analysis of Variance
AOAC Association of Official Analytical Chemists
Anon. Anonymous
A$^0$ Angstrom
@ at the rate
c.c. cubic centimetre
CD critical difference
CCD Central Composite Design
CCRD Central Composite Rotatable Design
CFTRI Central Food Technological Research Institute
CIRCOT Central Institute for Research on Cotton Technology
C$_h$ Coefficient of hulling
C$_{wk}$ Coefficient of wholeness of kernel
CIAE Central Institute of Agricultural Engineering
CLEA Cross-linked enzyme aggregates
CO$_2$ carbon dioxide
Ca Calcium
$^0$C Degree celcius
CV Co-efficient of Variance
d.b. dry basis
D.F degree of freedom
DM dry matter
e.g. Exempli gratia (for example)
Eq Equation
et al. et alii
etc. et cetera
FAO Food and Agricultural Organization
FMRC Farm Machinery Research Centre
Fig. Figure
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>hp</td>
<td>Horse Power</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>Sulphuric acid</td>
</tr>
<tr>
<td>i.e.</td>
<td>id est (that is)</td>
</tr>
<tr>
<td>ICAR</td>
<td>Indian Council of Agricultural Research</td>
</tr>
<tr>
<td>IIPR</td>
<td>Indian Institute of Pulse Research</td>
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<tr>
<td>Inst.</td>
<td>Institute</td>
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<tr>
<td>Int.</td>
<td>International</td>
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<tr>
<td>J</td>
<td>Journal</td>
</tr>
<tr>
<td>K</td>
<td>Potash</td>
</tr>
<tr>
<td>°K</td>
<td>Degree Kelvin</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kgf</td>
<td>kilogram force</td>
</tr>
<tr>
<td>kcal</td>
<td>Kilo calorie</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LSU</td>
<td>Louisiana State University</td>
</tr>
<tr>
<td>S.Em</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>m.c.</td>
<td>moisture content</td>
</tr>
<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µmol</td>
<td>micromole</td>
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<td>min</td>
<td>minute</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<td>mL</td>
<td>milliliter</td>
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<tr>
<td>mm</td>
<td>millimeter</td>
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<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>Sodium bicarbonate</td>
</tr>
<tr>
<td>NIR</td>
<td>Near Infra Red</td>
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NSP: Non Starch Polysaccharides
P: pressure
p: page
pH: Negative logarithm of the effective hydrogen ion concentration
Pvt.: Private
Res.: Research
rpm: revolution per minute
RSM: Response Surface Modelling
s: second
Soc.: Society
U: Enzyme unit
UV: Ultra Violet
w.b.: wet basis
β: beta
μ: micron
μm: micrometer
%: per cent
>: Greater than
Viz.: Namely
XYU: Units of Xylanase
CHAPTER I
INTRODUCTION

Pulses are the major sources of protein in Indian diet. The annual production of pulses in 2010-11 was 18.09 million tonnes from a cultivated area of 26.28 million hectares (Anon., 2011a). Among different countries producing pulses, India ranks first by contributing about 25.77% to the global pulse production (FAO, 2011). Pulses are a valuable source of proteins, minerals and vitamins in the daily diets of the people.

Pigeon pea (*Cajanus cajan* L.) is one of the important pulse crops of India contributing 20.87% to the total production of all pulses. India accounts for 90% of the total world production of pigeon pea (Goyal *et al.*., 2008). It is mostly consumed after dehulling in the form of dhal (degerminated split cotyledon). The total area and production of pigeon pea in Gujarat state during 2010-11 were 0.277 million hectares and 0.273 million tonnes, respectively. The average yield was 986 kg/ha (Anon., 2011b). In India, the annual production of pigeon pea in 2011-12 was 2.90 million tonnes from a cultivated area of 3.86 million hectares with the average yield of 751 kg/ha (Anon., 2011a).

Pigeon pea is significantly contributing to meet the dietary requirement of crude fibre, ash, fat, magnesium, manganese and copper. Pigeon pea contains high amount of vitamin B, Carotene and ascorbic acid (Singh and Diwakar, 1993). These are deficient in cereals; therefore, pigeon pea has a good supplemental value of cereal based diet. Pigeon pea is consumed as dehulled splits, whole, canned, boiled, roasted or ground into flour to make a variety of desserts, snacks and main dishes. The cotyledons of dry grains excluding seed coat are called dhal. Pigeon pea is mainly consumed as dhal because it takes less time to cook and has acceptable appearance, texture, palatability, digestibility, and overall nutritional quality.

Pigeon pea contains considerable amount of several anti nutritional factors, namely, protein inhibitors, amylase inhibitors and flab causing sugar and phytic acid. Pigeon pea contains some amount of polyphenolic compounds
(tannins) that inhibit the digestive enzyme-trypsin, chymotripsin and amylase. These are especially present in dark seed coated pigeon pea. These compounds create problems when pigeon pea is consumed in large quantities. Deshpande et al. (1982) reported that the dehulling legume grains may lower the tannin content and improve their digestibility. Dehulling pigeon pea helps to remove anti nutritional compounds such as polyphenols located in the seed coat.

The pigeon pea grain is considered as most difficult for dehulling as compared to other pulses owing to its seed coat which is more firmly attached with the cotyledons through a layer of gum and mucilage (Rout et al., 2007). Due to the presence of gummy layer and hard seed coat, it is difficult to dehull. The primary objective of dehulling is to remove seed coat from the cotyledons, during which four different fractions, i.e., dhal, broken, powder and husk are obtained. During dehulling, noticeable amount of cotyledon material and germ are removed resulting into considerable loss. In large scale processing of pigeon pea, the per cent loss of cotyledon in terms of powder and broken grain is as high as 12.8 % and 4.4 %, respectively (Singh, 1995). In view of this, when considering 10 % on an average milling loss of pigeon pea, it amounts to 2.7 lakh tonnes for all India with a value of ₹675 crores on dhal basis (Patel et al., 2001).

Pre milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion. There are many milling methods like wet milling, dry milling, CFTRI method, Pantnagar process, CIAE method and IIPR method developed for pigeon pea milling. There are various pre milling treatments, with respect to different milling methods, carried out before dehulling for loosening of seed coat of pigeon pea grain. In wet milling method, grains are soaked in water for 4 to 12 hours, then mixed with red earth and heaped for 16 hours. The grains are then dried for 2 to 4 days under sun in thin layer until the husk of all grains are loosened. Milling of pigeon pea on a commercial scale is generally based on dry milling technique. In dry milling, grains are passed through an emery coated roller for pitting. The pitted grains are mixed with linseed oil @ 1.5 to 2.5 kg/t followed by sun drying for 2-4 days.
After that the grains are sprayed with 3 to 5% water and heaped over night for tempering. In CFTRI method, the grains are conditioned by dry heat treatment in two passes using LSU dryer with 120 °C hot air. After each pass the hot grains are tempered in bins for 6 hours. In Pantnagar process, the pitted grains are mixed with 10% sodium bicarbonate solution. The treated grains are tempered for 4 hours in shade and then dried under sun to 9.5% moisture content. In CIAE method, scratched grains are soaked in tap water at ambient temperature for one hour. After draining the water, soaked grains are dried to 9 to 10% moisture content. In IIPR method, grains are soaked in water for 5 to 6 hours. The soaked grains are dried under sun drying for 1 to 2 days till grains start breaking under the teeth with sound, i.e., 9 to 10% moisture content.

All the above mentioned treatments are time consuming, require almost 4 to 7 days for the complete milling of pigeon pea. Also, the survey work of few pulse mills in Gujarat revealed that the dry milling treatments carried out during the pulse milling for pigeon pea take longer processing time, about 7 to 8 days depending upon weather as sun drying is required to get satisfactory milling after pre-treatment (Patel et al., 2001). But, all these pre-treatments do not permit easy removal of seed coat during the subsequent processing operation of pigeon pea milling. Moreover, these pre-treatments lead to higher processing cost, longer processing time and labour consuming for pigeon pea milling. It was revealed that the great potentiality of technology up gradation exists to get higher recovery of dhal as well as reduction in processing time and energy consumption (Patel et al., 2001).

This necessitated the suitable pre-treatment for pigeon pea milling that can shorten the processing time and improve the product quality. The price for the milled product is fixed on the basis of number of grains with intact husk (partly or wholly) in the sample, chipping of edges of the cotyledons, extent of surface scouring of the grain, and the variety of the pigeon pea. Dhal with a lesser or no husk, natural luster, yellow in colour and sharp edges of splitted cotyledons, can be sold in the market at a higher price.
At present, the consumers’ requirements are that the dhal should 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 (William et al., 1983). 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. Prolong cooking time results in a decrease in protein quality and a loss of vitamins and minerals. Hence enzyme treated pigeon pea dhal requires a detailed study which could reduce the cooking time of dhal.

It is necessary to have special pre-treatment to dissolve the glue that binds the cotyledons of pigeon pea grains to the seed coat. It is evident that dehulling quality is highly dependent on physical properties of grains and pre-treatments. Several grain characteristics affect the dehulling efficiency (e.g., size and shape of the grains, husk content and its thickness, adherence of the husk to the cotyledons, and moisture content). Interaction of pre milling treatments and grain characteristics play an important role in determining the dehulling quality. Selection of pre milling treatment also depends on the characteristics of the grain. Further, pre-treatments given to pigeon pea grains before dehulling considerably influence the cooking time. The cooking quality of pigeon pea is basically assessed by its cooking time (Singh et al., 1992).

The amounts of husk removed due to enzymatic pre-treatments on different aspects were reported by Verma, (1991), Bharodia, (2004), Deshpande et al. (2007) and Sreerama et al. (2009). The mechanism of enzymatic activity is governed by four interacting parameters, i.e., grain moisture content, enzyme concentration, reaction time and incubation temperature (Sarkar et al., 1998). Optimum levels of these parameters are necessary to get maximum recovery and better quality of dhal. Information on the effect of above parameters on dehulled fractions and cooking quality appears to be lacking. No well planned scientific study for optimization of hydrolysis of process parameters for better recovery of dhal has been attempted. Such an optimization is necessary for the industrial adoption of the
technology. Hence, it was considered necessary to optimize the parameters of enzymatic hydrolysis pre-treatment on different aspect, i.e., grain moisture, pH, enzyme concentration, reaction time (incubation time), incubation temperature of pigeon pea to get maximum recovery of dhal with good quality.

In view of above, the present research work was undertaken with the following objectives.

**Objectives**

To study the physicochemical properties of pigeon pea grains.

To study the effect of enzymatic pre-treatments on loosening of seed coat of pigeon pea grains.

To study the milling quality of enzyme treated pigeon pea.

To determine the effect of enzymatic pre-treatments on protein content of dhal obtained through different effective treatments.

To study the cooking quality of dhal obtained through different effective treatments.
CHAPTER II

REVIEW OF LITERATURE

The literature regarding the chemical composition of pigeon pea, physical characteristics, grain physical structure, dehusking machine, dry milling treatments, enzymatic treatments, protein content and cooking quality has been reviewed. The important reviews are given below under different sections.

2.1 Origin of Pigeon pea

The cultivation of pigeon pea (Cajanus cajan L.) crop goes back at least 3000 years. The centre of origin is most likely Asia, from where it travelled to East Africa and by means of the slave trade to the American continent. Today pigeon pea is widely cultivated in all tropical and semi-tropical regions of the World. Pigeon pea can be of a perennial variety, in which the crop can last 3–5 years (although the grain yield drops considerably after the first two years), or an annual variety more suitable for grain production. It is commonly known as arhar in Hindi, tuver in Gujarati and popularly known as red gram in English (Fuller and Harvey, 2006)

2.2 Proximate Composition

Tookey and Jones (1965) reported that there is a gum layer in between seed coat and cotyledons in different legumes. The pigeon pea grain contains 6.3% gum in layer between the seed coat and the cotyledons. Muller (1967) also reported the presence of thin gum layer in pigeon pea grains. Adherence of the husk to the cotyledons was possible due to the high lignin/gum content between the seed coat and cell walls of the cotyledons which acted as a binding substance. The husk of the pulses is attached to the cotyledons through a layer of gums (Kurien and Parpia, 1968; Siegal and Fawcett, 1976), the stickiness of which is due to the presence of calactomonus disaccharide, glucoronaic acid and glyco protein. The presence of these chemicals makes the
dehulling of pigeon pea a difficult process. Rachie and Roberts (1974) reported that the pigeon pea grain has a hard seed coat with slightly acrid taste.

Smartt (1976) reported that the pigeon pea is rich source of protein for animal and human consumption. It also supplies significant amount of minerals and vitamins. The pigeon pea grain contains about 19.2 % protein, 57.3 % carbohydrates, 1.5 % fat, 8.1 % fiber and 3.8 % ash. The chemical nature, quantity and level of hydration of gums affected the adherence and milling property of pigeon pea grains. Saxena (1985) reported that the testa contained cellulose, hemicelluloses, lipid, pectin and lignin that play an important role in adherence of seed coat to cotyledons.

Ramakrishnaiah and Kurien (1983) reported that pigeon pea grain contains about 11-14 % seed coat (hull), 2-5 % germ and the remainder is the cotyledon. The seed coat of pigeon pea grain is firmly attached to the cotyledons owing to uronic acid in the form of calcium pectate and compound structure of gums and mucilage in the pillar cells.

Narasimha (1984) found that the outermost layers (about 5%) of the cotyledon in pigeon pea are very rich in calcium (about 240 mg/100 g) and protein (nearly 40%). Scouring of 2–3% of this outer layer resulted significant loss of calcium and protein.

Faris and Singh (1990) reported that the pigeon pea composed of cotyledon 85 %, embryo 1.5 % and seed coat 14 %. The dietary nutrient quality of dry grain and dhal are summarized in Table 2.1.
Table 2.1 Dietary quality of pigeon pea

<table>
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<tr>
<th>Constituents</th>
<th>Dry grain</th>
<th>dhal</th>
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<tr>
<td>Protein (%)</td>
<td>18.8</td>
<td>24.6</td>
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<tr>
<td>Protein digestibility (%)</td>
<td>58.8</td>
<td>60.5</td>
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<tr>
<td>Trypsin inhibitor (units mg(^{-1}))</td>
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<td>13.5</td>
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<tr>
<td>Starch (%)</td>
<td>53.0</td>
<td>57.6</td>
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<tr>
<td>Starch digestibility (%)</td>
<td>36.2</td>
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<td>Amylase inhibitor (units mg(^{-1}))</td>
<td>26.9</td>
<td>-</td>
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<td>Soluble sugar (%)</td>
<td>3.1</td>
<td>5.2</td>
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<td>Crude fibre (%)</td>
<td>6.6</td>
<td>1.2</td>
</tr>
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<td>Flatulence factors (g 100 g(^{-1}) soluble sugar)</td>
<td>53.5</td>
<td>-</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.9</td>
<td>1.6</td>
</tr>
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<td>Calcium (mg 100(^{-1}) g dry matter )</td>
<td>120.8</td>
<td>16.3</td>
</tr>
<tr>
<td>Magnesium (mg 100(^{-1}) g dry matter )</td>
<td>122.0</td>
<td>78.9</td>
</tr>
<tr>
<td>Copper (mg 100(^{-1}) g dry matter )</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Iron (mg 100(^{-1}) g dry matter )</td>
<td>3.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Zinc (mg 100(^{-1}) g dry matter )</td>
<td>2.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

(Source: Faris and Singh, 1990)

Swami et al. (1991) reported that the cotyledon was rich in starch and protein and contained a water soluble polysaccharide mainly of arabinan type. The intermediate fraction had law starch than that in the cotyledon but it was rich in free sugars. Arabinogalactan type polysaccharides were found in the intermediate fraction which was gummy and hygroscopic. The alkali insoluble residue in the intermediate fraction was a complex of cellulose and non-cellulosic polysaccharides. The pectin content of intermediate fraction was greater in the difficult milling cultivar. The husk was rich in non starchy polysaccharides with varying amount arabinose and xylose. Both glucuronic acid and galacturonic acid were present in the husk whereas the cotyledon and intermediate fractions contained galacturonic acid only.
Singh and Diwakar (1993) reported that the pigeon pea contained considerable amount of several anti-nutritional factors, namely, protein inhibitors, amylase inhibitors, flab causing sugar and phytic acid. Pigeon pea contains some amount of polyphenolic compounds (tannins) that inhibit the digestive enzymes like trypsin, chymotrypsin and amylase. These are especially present in dark seed coated pigeon pea. These compounds create problems when pigeon pea is consumed in large quantities. However, the anti nutritional factors in pigeon pea are less than that in soybean, pea and common bean. Pigeon pea also contains some unavailable carbohydrates that reduce the bioavailability of other nutrients.

Oshodi et al. (1993) analyzed amino acids, fatty acids and mineral contents in pigeon pea. Amino acids analysis showed that the protein contained nutritionally quantities of most of the essential amino acids but was deficient in sulphur containing amino acids. The total essential amino acids in the pigeon pea is 43.61%. Linoleic and palmitic acids were predominant fatty acids with quantities as high as of 54.8 and 21.4%, respectively in the oil sample of pigeon pea. Caprylic, lauric, oleic and eicosenoic acids were present only in small quantities. The results also showed that pigeon pea is rich in potassium, magnesium and calcium while it was deficient in sodium.

Padmanabhan et al. (2009) investigated the nutritional quality of dehusked whole grains (gota) and dehusked splits (dhal) in red and white varieties of pigeon pea regarding proximate composition and certain lipid soluble bioactive components. A decrease in fat and crude fiber was noticed when gota was converted to dhal. The lipid profile of gota and dhal from red and white husk pigeon pea types indicated that essential fatty acids were more in gota than in their respective dhal. Gota from white husk variety contained more tocopherols than in the red variety. Dhal contained less tocopherols than gota. Cooking time and dispersed solids on cooking indicated good cooking quality of gota. The results indicated the nutritional superiority of gota over dhal and its similarity with dhal in cooking characteristics.
Powar et al. (2009) determined the proximate compositions of pigeon pea grains, viz., moisture, carbohydrate, protein, fat, crude fiber and ash on dry weight basis as shown in Table 2.2.

**Table 2.2 Proximate composition of pigeon pea grains**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Proportions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %, (w.b.)</td>
<td>9.94</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>54.05</td>
</tr>
<tr>
<td>Protein %</td>
<td>21.28</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.15</td>
</tr>
<tr>
<td>Crude fiber %</td>
<td>8.44</td>
</tr>
<tr>
<td>Ash %</td>
<td>4.13</td>
</tr>
</tbody>
</table>

(Source: Powar et al., 2009)

### 2.3 Physical Properties

Several grain characteristics affect the dehulling efficiency (e.g. size and shape of the grains, husk content and its thickness, adherence of the husk to the cotyledons and moisture content). Interaction of pre milling treatments and grain characteristics play an important role in determining the dehulling quality. Selection of pre milling treatment also depends on the grain characteristics.

Grain size is one of the factors affecting the dehulling process in pulses. Ehiwe and Reichert (1987) further suggested that uniform and medium-sized pigeon pea grain would improve the efficiency of dehulling. Very small to small grains are more difficult to dehull because they split even before removal of husk and, hence, require several recycling steps and, therefore, are not generally preferred by millers.

Similar to grain size, grain shape also plays a vital role in the selection of dehulling devices (Kurien, 1977). Although, round grains are considered better for dehulling (Singh and Jambunathan, 1990) while very angular grains lose excessive amounts of cotyledon material during dehulling. Sharper edges are preferentially lost from the angular grains, whereas, more grain mass is
removed from flatter grains. As a result, flatter the grains, higher the amount of powder and broken (i.e. small pieces of cotyledons). In addition, round shaped grains split more readily than flatter grains, thus improving the efficiency of dehulling and splitting (Kurien, 1984).

Singh and Oswalt (1992) reported that the colour of the grain ranges from silver, white, cream, fawn, black, pink or red to purple. They are blotched or speckled. Pigeon pea 100 grain mass ranges from 2.8 to 22.4 g. However, the varieties mostly cultivated have the 100 grain mass ranging from 7.0 to 9.5 g. Grain shapes are oval, pea-shaped, square or elongate. The most common is a pea shaped grain found in large seeded late varieties.

Singh et al. (1992) determined physical characteristics of BDN 2 variety of pigeon pea grains. They found cream seed coat colour, 11.0 % moisture content, 7.7 g /100 grain mass, 6.2 ml grain volume, 6.4 % floatation value, 1.08 g/g swelling capacity, 17.3 kgf grain hardness and 14.8 % husk.

Bhandari et al. (1994) studied the physical properties such as single grain mass, grain surface area and volume of 33 determinate and 72 indeterminate genotypes of pigeon pea. In the determinate group there was a strong positive relationship ($R^2 = 0.93$) between grain surface area and volume. Sixteen determinate had spherical grains, 14 oval and 3 rectangular. In the indeterminate group grain, shape was spherical in 32 genotypes, ellipsoidal oval in 31 and rectangular in 9. They also noted that even within a particular genotype there were variations in grain mass, volume and surface area.

Umbarkar et al. (1998) selected seven pigeon pea cultivars, commonly grown in India, for determination of the physical properties and milling characteristics. The influence of physical properties on milling behaviour of pigeon pea grains was assessed and correlated. Physical properties varied between cultivars. Improved milling procedures including soaking seeds in 6 % sodium bicarbonate rather than in water gave increased dhal recovery in improved cultivars but not in two local cultivars.

Baryeh and Mangope (2003) evaluated the physical properties of pigeon pea as a function of grain moisture content varying from 5 to 25 % (w.b.). They
observed grain surface area, volume and sphericity decreased nonlinearly from 100.28 to 47.39 mm$^2$, 94 to 28 mm$^3$ and 0.91 to 0.82, respectively, while true density, bulk density and porosity increased nonlinearly from 0.75 to 1.22 g/mm$^3$, 0.70 to 0.87 g/mm$^3$ and 8 to 28 %, respectively. Thousand grain mass, angle of repose and terminal velocity increased linearly from 96 to 140 g, 17$^0$ to 31$^0$ and 6.52 to 11.58 m/s, respectively. The coefficient of static friction increased nonlinearly from 0.28 to 0.51 for plywood, 0.23 to 0.38 for galvanized steel and 0.18 to 0.31 for aluminium.

Mangraj et al. (2005) determined physical and gravimetric properties of pigeon pea at 9.53 % average moisture content (w.b.). Average physical and gravimetric properties were evaluated as length 6.14 mm, breadth 4.9 mm, thickness 4.41 mm, equivalent diameter 5.10 mm, bulk density 0.885 g/ml, true density 1.389 g/ml, porosity 36.1% , sphericity 0.811 and 1000 grain mass 95.73 g.

Jain and Doharey (2009) determined physical properties of brown and red variety of pigeon pea. They observed average value of different parameter like moisture content 10.1±0.23 and 10.0±0.56 %, equivalent diameter 5.5±0.12 and 4.4±0.21 mm, sphericity 0.87±0.10 and 0.81±0.14, bulk density 910±0.67 and 861±0.58 kg/ m$^3$, true density 1360±0.34 and 1271±0.22 kg/m$^3$, porosity 33.1±0.53 and 31.2±0.41 %, husk content 12.5±0.25 and 14.0±0.12 % for brown and red variety, respectively.

Singh and Kotwaliwale (2010) determined physical properties of pigeon pea as a function of moisture content. The average length, breadth and thickness increased linearly from 6.1 to 6.5 mm, 5.4 to 5.8 mm and 3.8 to 4.4 mm, respectively with increase in moisture content from 10 to 30%. The sphericity increased linearly from 0.82 to 0.84. The grain volume and surface area increased linearly from 52.6 to 70.4 mm$^3$ and from 69.5 to 74.3 mm$^2$, respectively with increase in moisture content. The true density and bulk density decreased linearly from 1299 to 1203 kg/m$^3$ and 830.3 to 762.9 kg/m$^3$, respectively. However, no definite relationship was observed between porosity and moisture content.
2.4 Dehusking Machine

Traditional technologies and machines have several limitations and difficulties. Therefore, attempts have been made either to improve upon the traditional methods and machines or to develop new ones. Although, certain machines developed in other countries for the cereals and millets have also been used for dehulling grain legumes. The recovery of dehulled grains is not as much as in traditional methods (CFTRI, 1977). Moreover, the dehulling of grain legumes, particularly pigeon pea, can be efficiently and economically done in the two general steps of pre milling treatments to loosen husk and dehulling followed by splitting.

A suitable technology and machinery has been developed at Central Food Technological Research Institute, Mysore, India, for dehulling pigeon pea and other grain legumes. The husk of pigeon pea could be loosened and made brittle and pulverizable if treated under appropriate temperature conditions and dried to a critical moisture level. The temperature conditions and critical moisture level vary from one cultivar to another, but satisfactory milling characteristics could be imparted to the most difficult-to-mill ones if they are pretreated under proper conditions. The grain can be hardened and the extent of splitting reduced considerably by reducing the moisture content and thereby, scouring losses.

Attrition type dehullers and mills are particularly suitable for dehulling and splitting legume grains with loose seed coats whereas, abrasive type dehullers are suitable for dehulling grains with more tightly adhering seed coats (Kurien, 1984).

Sahay and Bisht (1987) studied the effect of surface roughness (carborundum grade number) on milling efficiency and observed milling efficiency as 91.67% for pigeon pea, when 30 grade grit size was used.

Sahay and Bisht (1988) developed a carborundum roller cylindrical mill to dehusk and split pulse grains to make dhal. The clearance between the outer screen cage and inner carborundum grit coated abrasive roller was maintained 10 mm throughout. It facilitated even abrasion of the grains. The effective capacity was 100 kg/h and could be owned, operated and manned by less
skilled people. Performance of the mill was evaluated for different pulses at different rotational speed and feed rates. It was found that maximum milling efficiency (88%) was obtained at 850-900 rpm (13.5-14 m/s roller surface speed). Dhal recovery was 74-75%.

Sahay (1990) developed a general purpose abrasive mill to pearl and dehusk cereals and pulses. It incorporated integral milling and cleaning chambers and a grinding wheel rotor, driven by a 3.7 kW electric motor. Its capacity at maximum dehulling efficiency of 75% was 96-340 kg/h, depending upon the grain being handled. The cost of processing varied from 4.5 to 9.2 per tonne of feed. It could be owned, operated and manned by small scale processors in rural and semi-urban areas.

Phirke et al. (1992) developed a mini dhal mill running on 1.0 hp single-phase electric motor with four units, viz. splitting sieve, aspirator and polisher in order to obtain polished dhal from pigeon pea grains. By changing sieves, the same unit can be used for other pulses. The performance of a mini dhal mill was evaluated in the laboratory and the results are presented. The maximum milling efficiency was observed at 4.25 mm disc clearance with 45 kg/h capacity for pigeon pea. A similar value for green gram and black gram was obtained at 60 kg/h capacity. The respective recoveries obtained were 73.75, 85.50 and 84.63%.

Mandhyan and Jain (1993) studied the effect of peripheral speed, clearance between rotor and concave using two cultivars. For ‘Godarwara’ (Small grain cultivator), the optimum peripheral speed was 6.51 m/s at concave clearance of 10.43 mm and the corresponding figure for ‘Patan’ (medium grain cultivar) were 6.75 m/s and 11.74 mm, respectively with the splits recovery of 73.85% and 71.24% for the said varieties.

Phirke et al. (1996) studied the dehulling behaviour of two pigeon pea cultivars with respect to the dehulling index, using response surface modeling and a tangential abrasive dehulling device. Rotor speed and surface roughness (emery grade number) were optimized for minimum dehulling time and maximum dehulling index with two pre-treatments, viz., (i) urea treatment using 8 % solution in 1:25 proportion with grain for 5 h and (ii) heat treatment after
urea application in hot air oven at 60 °C for 5 min after stabilization of temperature. The results revealed that, irrespective of pre-treatments and cultivars, the optimum rotor speed and emery grade were 12.6 m/s and No. 30, respectively. However, hot milling reduced the dehulling time to about 50% for both the cultivars, and enhanced the dehulling index by about 8% for `C 11' and 6% for `No. 148' cultivars. Dehulling characteristics and end products of hulling were determined at different normal loads on grain holding above factors at optimum levels. The maximum product recovery was obtained at a loading of 75 g for `C 11' and 100 g for `No. 148' cultivars.

Srivastava (1997) reported the use of slotted screen in the dehusking machine. It was mounted around the emery coated cylinder in the dehusking machine for milling of pigeon pea grains. As the hopper opening increases from 15 mm to 25 mm, the hulling efficiency decreased from 64.04 % to 43.23 % and correspondingly throughput capacity increased from 10.13 kg/h to 78.43 kg/h.

Kumar et al. (2004) studied the effect of machine parameters, i.e., roller speed (12 to 16 m/s), roller inclination (4.5 to 6.5 degree) and roller length (22.5 to 37.5 cm) on pigeon pea milling and to optimize the maximum percentage of finished product and hulling efficiency using response surface methodology. The multiple second order model for finished product percentage and hulling efficiency represented data very well. The optimum values of these machine parameters for the experimental set-up (emery roller dhal mill) with roller diameter of 15 cm were found to be 15.50 m/s, 5.14 degree and 33.58 cm, respectively for maximum percentage of finished product (81.09) and highest hulling efficiency (95.24%) for the pigeon pea milling.

Lal et al. (2004) developed a low capacity (85-125 kg/h) pulse dehusking and splitting mill, named as IIPR Dhal Chakki at the Indian institute of Pulses Research to meet pulse processing demand of small millers. This is a vertical burr mill, using stationary rubber and rotating steel discs as abrasive surfaces, devised to reduce scouring losses to cotyledons. The performance of the mill was evaluated for pigeon pea with three different pre milling treatments, for loosening of husk. Sodium bicarbonate treatment gave the maximum finished product recovery of 76.48% as compared to 71.86% obtained from oil and
water treatment. Low breakage and powdering were important features of the mill.

Jain and Doharey (2009) fabricated a pulse milling unit consisting of tapered abrasive roller and a centrifugal splitter for milling pulses at rural level. The unit was evaluated at laboratory scale with two cultivars of pigeon pea. It was observed that 75.3 to 76.8 % head dhal recovery could be obtained by milling after oil and water treatment of pulse. A pilot scale trial with one ton pigeon pea was also undertaken to work out the feasibility of the system. The milling system worked well even at higher scale with 76.7 % head dhal recovery. The capacity of machine was 70 kg/h for pigeon pea. The dhal milling system could be easily operated with little training.

Mangaraj and Singh (2011) optimized the independent milling parameters of CIAE (Central Institute of Agricultural Engineering, Bhopal, India) dhal mill, i.e., roller speed, emery grit size and feed rates. These parameters were optimized for pigeon pea, chickpea and green gram dehulling using response surface methodology (RSM) and Central Composite Rotable design (CCRD). The roller peripheral speed of 10 m/s, emery grit size 0.3 mm and feed rate 101.60 kg/h were found optimal. The maximum pulse recovery on the optimized independent parameters was observed for pigeon pea as 76.36 % (77.04 % predicted) followed by chickpea as 73.80 % (73.06 % predicted) and green gram as 71.25 % (69.82 % predicted). The maximum milling efficiency on optimum machine parameters was observed for pigeon pea as 83.0 % (82.79 % predicted) followed by chickpea as 74.80 % (75.53 % predicted) and green gram as 78.0 % (78.24 % predicted).

2.5 Standards for Milling Quality

Narasimha et al. (2003) reported the milling quality of pulses. The price for the milled product is fixed, depending on number of grains with intact husk (partly or wholly) in the sample, chipping of edges of the cotyledons, extent of surface scouring of the grain, and the variety of the pulse. There is a substantial difference in the prices of first, second, and poor quality dehusked splits, which is a matter of much concern to millers as well as to the consumers.
2.5.1 Milling characteristics of pigeon pea

Several factors influence the milling characteristics of pigeon pea and these must be considered in developing suitable pre-milling treatments to loosen the husk, i.e., the content of husk and its hardness; the amount, chemical nature, and hydration level of the gums; the shape, size, and moisture content of grains, hardness or softness, etc. (Kurien, 1977). The seed coat must be properly conditioned, for easy removal in the machine with a minimum loss of grain. In a study of physical properties and milling characteristics of several varieties of pigeon pea, scientific explanations of some of the properties exhibited by this grain during dehusking have been found.

2.5.2 Husk and cotyledon content

The husk content of pigeon pea varieties studied varied from 10.5% to 15.5%. With the low germ content (0.6-1.4%), the cotyledon content was found high in varieties with low husk content and vice versa. However, there was no definite correlation between the grain size and husk content of a variety. Big, bold grains had both high and low husk contents, e.g., varieties like S-141-31 (14.8%) and HY-3A (10.5%). However, the smaller grains generally had a higher content of husk.

The husk is attached to the cotyledons by a layer of gums and lignin (Kurien, 1971). The adherence of the husk to the cotyledons depends on the thickness of these gums, which in turn depends on the amount, chemical nature, and extent of hydration.

2.5.3 Dehulling moisture content

Ramakrishnaiah and Kurien (1983) studied the optimum moisture content for dehulling of pigeon pea. They reported that the dehulling efficiency was decreased with increase in moisture content. This reduction was not significant up to 10% moisture content (w.b.). Ehiwe and Reichert (1987) reported that the moisture content of grain played an important role in dehulling.
They observed coat breaking of field peas was affected by grain moisture followed by temperature and cultivars.

Saxena et al. (2007) reported that the soaking pigeon pea grains in 6 % sodium bicarbonate solution for 1 h, followed by oven drying to 10 % moisture. The pre milling treatment resulted into 94 % yield of dhal. Treatment reduced gum and pectin content, increased enzyme activity, but caused losses in protein and starch content of the dhal.

Goyal et al. (2008) studied the effect of dehulling time, moisture content and the mustard oil (as pre milling treatments) on dehulling efficiency and losses. They optimized this parameters using response surface methodology. A quadratic model satisfactorily described the dehulling efficiency with high coefficient of determination R² (0.95). It predicted a maximum dehulling efficiency of 83.2 % at 10.1 % moisture content, 12.3 s dehulling time and 0.3 % mustard oil treatment. Moisture content and dehulling time affected dehulling loss significantly whereas the effect of mustard oil treatment was non significant. Dehulling loss of 2.5 % was predicted at optimum conditions. Dehulling efficiency and loss at optimum conditions were observed to be 82.4±0.8 % and 3.1±0.4 %, respectively and these values were close to the predicted values.

Goyal et al. (2009) studied the effect of grain moisture content on pitting. Pitting of the grain was done at 6, 8, 10, 12 and 16 % moisture content using dhal mill developed by central Institute of Agricultural Engineering, Bhopal (India). Grains having a cracked hull or partly dehulled varied from 35.3 to 85.3 % during pitting with maximum value at 10 % moisture content and reduced with an increase in moisture content.

2.6 Dry Milling Treatments
Kurien and Parapia (1968) described the treatments as the pitted grains were mixed with about about 1 % oil and spread in thick layer for sun drying for 2 to 5 days. Grains were heaped during the night to preserve the heat. About 2 to 3 % of water was added to the grains which were subsequently passed through the
roller for dehusking. In this process about 40 to 50 % of the grains were dehusked.

Saxena (1985) reported proteins of the lipid membrane either lie on the periphery of the lipid bi-layer or impregnated within it. Entry of oil may displace the proteins from the surface of the bi-layer causing the loosening of the membrane. This will loosen the binding force between husk and cotyledon. Polar group of oils may interact with the cation present in the vicinity of the membrane and cause loosening of the husk. Drying of oil-water treated grain may cause the formation of the cavity between husk and cotyledon.

Makhoha (1992) studied processing of pigeon pea. Cleaned and graded grains were treated with addition of edible oil at 0.35 % by weight. The grains were then sun dried for 8 to 9 h followed by preliminary dehulling using a dehuller. The grains were then tempered with addition of water at 8 % by mass, equilibrated in a heap for 3 h and dried in the sun for 4 h. The tempered grains were dehulled that gave 70 % recovery as dhal.

Singh and Diwakar (1993) reported that cleaned and graded pigeon pea is passed through an emery coated roller for pitting. The pitted grains were manually mixed with warm oil (about 1 % linseed or mustard oil) followed by sun drying for 2-5 days. On penultimate day of sun drying, 2-5 % water was sprinkled on the grains and it is thoroughly mixed and heaped overnight for tempering.

Perera (2000) studied the importance of physical properties of grains and pretreatments given to pigeon pea grains in order to get quality dhal with high recovery rate. Two oil treatments (coconut and sunflower), chemical treatment (1 % aqueous solution of bicarbonate) and control (sun dried) were employed to study the recovery rate and dhal quality of three newly developed varieties (MPG537 MI12, ICPL87, and ICPL90050) and one earlier recommended variety ‘Prasada’ by using FMRC dhal processing machine. Dhal yield of pigeon pea ranges between 59-63 % for sunflower oil, 63-71 % for coconut oil, 56-65 % for 1 % aqueous solution of bicarbonate and 41- 59 % for control. Considering variability in dhal yield (41-71 %) and its quality, it was observed that amongst the different pre-treatments, coconut oil was found to be the best.
Further, the recommended variety ‘Prasada’ gave high quality dhal with favourable characteristics.

Patel et al. (2001) reported the status of pigeon pea milling industries in Gujarat. It was reported that the major change found in the presently followed system and the traditional method was the drying step after pitting and oil treatment. In the old method, after every pitting operation edible oil treatment was given and the grains were exposed to sun drying for one day. Then next day pitting continued till all the grains got dehusked. On the other hand, in the new process, the sun drying was not carried out after pitting and oil treatment. But after mustard oil treatment, the grains were stored in bin for about 36 h which lead to loosening of husk. Then next day further pitting was carried out.

Deshpande (2003) treated 10 kg pigeon pea grains with 20, 30 and 40 g oil with 3, 4 and 5 % water application without scratching operation. This gave dhal recovery of about 17 to 29 %, whereas the scratched and treated sample gave the dhal recovery of 58 to 67 %.

Deshpande et al. (2007) conducted oil-water treatment included scratching of grain followed by mixing soyoil @3 kg/tonne. Oil mixed grains were then held for 8-10 h. After that, grains were kept for 3 - 4h for equilibration. Then water was mixed (4%) and grains were heaped overnight. Next day grains were dried in the sun or by mechanical dryer to bring down moisture content to 10 % (w.b.). Satake grain testing mill TM05 (Satake Engg. Ltd., Japan) was used for conducting milling experiments. Soyoil-water treatment showed dhal recovery of 67.61 to 70.39 %.

Goyal et al. (2009) optimized hulling efficiency and hulling losses for pigeon pea. Effects of hulling time, moisture content and cottonseed oil as pre-milling agent were studied and optimized using response surface methodology. A quadratic model satisfactorily described the hulling efficiency with an $R^2$ value of 0.93. It predicted maximum dehulling efficiency of 89.98 % at 9.82 % moisture content (w.b.), 12.05 s time of hulling and 0.28 % cottonseed oil treatment. Linear model developed for hulling loss showed significant effect of time of hulling whereas effect of oil treatment and moisture content were non-significant. The model predicted hulling loss of 2.92 % at optimum conditions.
The results of the model were validated experimentally and were within the range.

2.7 Enzyme Treatment

Verma (1991) investigated the effect of enzyme hydrolysis on the milling of pigeon pea. Pigeon pea grains were treated with enzyme, obtained from Aspergillus fumigatus. The hydrolysis parameters were (i) temperature during hydrolysis (32.7, 40, 45, 50 and 53.7 °C) (ii) incubation period (4.6, 9, 12, 15 and 19.4 hours) and (iii) moisture content during hydrolysis (12.7, 20, 25, 30 and 37.3 % w.b.). It was observed that hulling efficiency of treated grains increased by 18.86 % and 13.08 % as compared to untreated and water treated grains, respectively, both at milling moisture content of 10 % (d.b.). It was concluded that the maximum hulling efficiency (88.9 %) could be obtained at 26.6 % (w. b.) moisture content of grain during hydrolysis, 0.08: 260 enzyme protein grain ratio and 46.7 °C incubation temperature for the period of 12.7 h.

Saxena et al. (1993) used food grade mixed activity enzymes (i.e. xylanase and cellulase) as husk loosening agent. He reported a maximum hulling efficiency of 88.93 % at an enzyme concentration of 0.08 g protein per 260 g pigeon pea grain. Grains were treated with the enzyme and allowed to incubate. During this period of incubation, enzymatic hydrolysis took place which brought about the biodegradation of complex molecules of the grain. The complex gums were degraded which resulted in easy dehusking. It established that a lesser force was required to bring about the dehusking of enzyme treated grain. The action of enzyme also disturbed the microstructure of the grain affecting its strength. They further reported an increase in the protein digestibility and 37.03 % reduction in cooking time. Further, this dhal was reported to cause less gastritis due to fermentation which broke down the polysaccharides responsible for causing gastritis in many people.

Zambre (1994) reported a decrease in gum content after enzyme treatment. The protein digestibility of the treated dhal was more than that of untreated dhal. He also reported that enzyme treatment caused grain to split at a lesser
force and deformation. This was due to change in microstructure which affected the strength of the grain.

Benamrouche et al. (2002) studied the effect of (1→4)-β-endo-xylanase treatment on wheat bran. By using UV fluorescence microscopy, this study confirmed the degradation of the aleurone cell wall after (1→4)-β-endo-xylanase treatment. After 24 h incubation, the aleurone layer was completely lost. However, the tissues in the outermost layer of the bran retained their integrity during xylanase treatment. They also reported that 80 % and 51.8 % of the total carbohydrate was liberated by the hydrolysis of aleurone and inner bran respectively, whereas no carbohydrate was released by (1→4)-β-endo-xylanase treatment.

Deshpande (2003) treated 60 kg pigeon pea grains with 4 % soy oil and 4 % CIRCOT enzyme. The samples treated with soy oil and enzymes were mixed thoroughly to achieve uniform application of enzyme to the grains. The treated grains were then pitted. These samples were then soaked in water for varying duration, i.e., 45, 60, 75 and 90 minutes followed by drying to 10 % moisture content. The results indicated the dhal recovery in the range of 81.11 to 84.58 % for 75 minutes subsequent soaking compared to other soaking treatments.

Bharodia (2004) studied on enzymatic pre-treatments for loosening of seed coat of pigeon pea grains. The enzyme solution soaking treatment having 1000 ml soaking volume, 0.05 g enzyme concentration for 2 kg pigeon pea with 7 h soaking time was found the most effective considering the quality of dhal. The amount of husk removed was to the tune of 76.24 %.

Arora et al. (2007) studied the optimum process parameters for milling of enzymatically pre treated rice. To obtain a higher quality of finished product (polished white rice), three process parameters (enzyme concentration 0.0015 g/ml - 0.0055 g/ml, incubation time 1-3 min and incubation temperature 27-47 °C) were examined and optimized for developing an efficient milling system. The data analyzed according to response surface methodology (RSM) showed that with enzymatic pre-treatment, the rice bran layer softened up and
removed easily in the mechanical polisher. Optimum process parameters for minimum percentage of broken and good cooking quality were found as 0.0015 g/ml of enzyme concentration, 40 °C of pre-treatment temperature and 2 min of pre-treatment time.

Deshpande et al. (2007) conducted pre milling trials on pigeon pea grain employing soy oil water and CIRCOT microbial consortium enzyme treatments. Application of CIRCOT microbial consortium treatment included treating grains with 4% consortium. The consortium was mixed in water and grain samples were soaked for 3 h. The treated samples were kept for equilibration for 3 h, and then dried to 10% moisture content. They observed dhal recovery of 67.61 to 70.39 % and 71.39 to 73.85 %, respectively in comparison to dhal recovery of 63.19 to 66.27 % by conventional dhal milling method.

Sreerama et al. (2009) evaluated the xylanase and protease pre-treatments on the dehulling properties of green gram, black gram, red gram and horse gram. Xylanase-mediated degradation of non starch polysaccharides (NSP) had facilitated the easy dehulling of green gram, black gram and horse gram. Xylanase pre-treatment of horse gram resulted in 84.4 % dehulled grains, whereas 78.4 % and 75.7 % dehulled grains were produced in green gram and black gram, respectively. However, protease pre-treatment was more efficient in improving the dehulling properties of green gram and black gram in addition to red gram with higher amount of dehulled grains (> 78 %) and lower amount of fines. Selective improvements in the degree of dehulling, dehulling index and dehulling efficiency were observed in enzyme treatments compared to buffer and oil treated controls.

Yoo et al. (2009) examined the effect of cell wall degrading enzymes added to temper water on wheat milling performance and flour quality. An enzyme cocktail consisting of cellulase, xylanase and pectinase and five independent variables (enzyme concentration, incubation time, incubation temperature, tempered wheat moisture content and tempering water pH) were manipulated in a response surface methodology (RSM) central composite design. A single pure cultivar of hard red winter wheat was tempered under defined conditions
and milled on a Ross experimental laboratory mill. Some treatment combinations affected flour yield from the break rolls more than that from the reduction rolls. However, a maximum flour yield was not found in the range of parameters studied. Though, treatments did not affect the optimum water absorption for bread making, enzyme-treated flours produced dough exhibiting shorter mixing times and slack and sticky textures compared with the control. Regardless of differences in mixing times, specific loaf volumes were not significantly different among treatments. Crumb firmness of bread baked with flour milled from enzyme treated wheat was comparable to the control after 1 day but became firmer during storage up to 5 days.

2.8 Scanning Electron Microscope

Mayande (1987) examined the effect of water treatment using scanning electron microscope and reported that in water and sodium bicarbonate treatment, damage took place in the gummy layer resulting in loosening of husk from the cotyledon.

Saxena et al. (1990) conducted electron microscope studies on sodium bicarbonate treated pigeon pea grains to investigate the effect on loosening of binding forces between the seed coat and the cotyledon. Soda solution in 3 concentrations (4, 6 and 10%) was prepared and mixed with the grain in 3 ratios (20:1, 10:1 and 6.67:1 kg/litre). Electron microscope studies conducted on treated grain confirmed the effect of soda treatment in loosening/rupturing the seed coat of the pigeon pea grain. Experiments conducted on a standard laboratory mill indicated that a maximum dehusking efficiency of 90.16% was obtained for the grains treated with 10% soda solution at 20:1 ratio and tempered for 5 h at room temp.

2.8.1 Grain physiology

Mayande (1987) conducted electron microscopic studies of pigeon pea seed coat and observed three layers (i) outer layer of sclerides (ii) pillar cells and (iii) an unknown layer which envelops the cotyledons. The sclerides look like longitudinal fibrous cells. Pillar cells mainly linked the sclerides and the third unknown layer. It was reported that this layer might be of gums and mucilages.
Below this layer lie the granular structures of starch of different sizes in which the interspaces were filled with protein bodies. Using electron microscopic photographs, it was demonstrated that the protein bodies lie near the periphery of cotyledons. The cross section of pigeon pea grain is shown in Fig. 2.1

![Cross section of pigeon pea grain](image)

**Fig. 2.1 Cross section of pigeon pea grain**

### 2.9 Protein Quality of Pigeon pea dhal

Singh and Eggum (1984) reported the factors affecting the protein quality of pigeon pea (*Cajanus cajan* L). Among important food legumes, pigeon pea contained lowest amount of limiting sulphur amino acids, methionine and cystine implicating the importance of these amino acids in protein quality improvement programme. Large variation existed in the levels of protease inhibitors of pigeon pea varieties. The concentrations of these inhibitors were significantly higher in some of the wild relatives of pigeon pea. Protein digestibility of cooked pigeon pea meal remained low and this could be due to the presence of certain compounds other than trypsin inhibitors. Pigeon pea polyphenolic compounds adversely affected the activity of digestive enzymes and this affect the protein quality of pigeon pea.
Srivastava et al. (1999) assessed the change in protein content both in cotyledon (manually dehulled) and finished product (dhal) obtained by different pre-treatments (water soaking, water spray, oil treatment, sodium bicarbonate treatment and enzyme treatment). All the pre milling treatments except sodium bicarbonate treatment caused significant loss in protein content in cotyledon over untreated sample. Oil treatment resulted in maximum loss (3.18 %). Protein contents of dhal ranged from 20.71 to 22.45 %. Maximum protein content was observed in enzyme treated sample and minimum in oil treated sample.

2.10 Cooking Quality

Dehulling helps to reduce cooking time. Before consumption, cooking is the most important household practice followed for pulse. Cooking of pulses is a complex phenomenon controlled by several factors. Softening of pulses during cooking occurs through a reaction of phytate, present as sodium / potassium phytate in the cotyledons, with the insoluble calcium/ magnesium pectinate present in the cell walls. This reaction converts the Ca/Mg pectinate to the soluble Na/ K pectate (Muller, 1967). Heat treatment during cooking helps in loosening the intercellular matrix of the middle lamella sufficient to allow separation of individual cells (Rockland and Jones, 1974). Cooking quality of dhal is a function of the duration of cooking, i.e., the time required to attain desired softness. The cooking quality is also judged to some extent on increase in volume after cooking, higher dispersibility of solids into cooking medium and improved texture after cooking from consumer’s points of view (Silva et al., 1981)

Cooking time of whole pulses varies from 60 min (for pulses such as green gram and lentil) to 150–200 min (for pulses such as beans, horse gram and soybean). Cooking times, which were reduced by almost 50% by mere dehusking and cooking time of various dhal range from about 20 to 100 min (Narasimha, 1984).

The cooking quality of dehusked splits is also influenced by the dehulling method, in particular, by the pre milling treatments. However, there may not be
a direct influence of the dehulling machines on the cooking time of dehusked splits. It is not the mechanical action of the roller machine or disk shellers that influence the cooking time but the pre-treatments given to grains before dehulling that considerably influenced the cooking time, as observed in dehulled pigeon peas (Singh, 1987).

Singh and Rao (1995) studied on quick cooking dhal of pigeon pea as influenced by salt solution and enzymatic pre-treatment. Quick cooking dhal of pigeon pea was prepared by employing various salt solutions and enzymatic treatments. Sodium bicarbonate was effective in reducing the cooking time but the quality was affected. Pectinase treatment decreased the cooking time as compared to other enzymes and salt solution. Generally, the acceptability score of dhal was the highest for pectinase treated dhal followed by the control, solution of sodium bicarbonate and salt mixture.

Vijyakumari et al. (1997) reported the poor cooking quality of dhal prepared by wet method. This was especially true in case of the pigeon pea for which cooking time increased with duration of soaking. However, such dhal had an attractive appearance and a more desirable flavour.

Saxena and Srivastava (1998) evaluated cooking time of dhal obtained with different pre milling treatments of pigeon pea on a laboratory mill. It was found that the dhal obtained from enzyme treated sample took 3 minute less time in cooking over the control. Water soaking method resulted in hard to cook dhal, which nearly took 15 minutes more time of cooking.

Singh (1999) studied the cooking quality of pulses. It was reported that the pre-treatment reduced the cooking time of pulses when soaked in sodium bicarbonate solution, ammonium carbonate, trisodium phosphate, enzymepectinase, sodium tripolyphosphate, sodium chloride, sodium carbonate, citric acid and salt mixed with sodium bicarbonate.

Singh et al. (2000) examined the cooking qualities of six pulses, namely, chickpea, pigeon pea, mung bean, urd bean, lentil and field pea as influenced by dehulling, soaking solution and enzyme treatment. The pre-treatments of soaking in NaHCO₃ solution and pectinase significantly reduced the cooking
time in both whole grain and dhal components. Sodium bicarbonate solution was more effective than the enzyme treatment in reducing cooking time in whole grain, whereas the latter was more effective in dhal samples. Effect of pectinase enzyme treatment was most pronounced in pigeon pea followed by field pea and chickpea in dhal samples. Soaking in NaHCO$_3$ solution resulted in the highest reduction of cooking time of chickpea and the lowest in pigeon pea in case of whole grain sample.

Fasoyiro et al. (2005) evaluated physical characteristics, cooking and sensory characteristics of three varieties of pigeon pea. Swelling capacity and cooking time ranged between 45.7 to 54.7 % and 170-210 min, respectively. The red brown coloured variety was most acceptable amongst three varieties by sensory analysis. Soaking and cooking method reduced cooking time but most of the processing methods reduced protein and mineral contents of pigeon pea. Roasted and fried grains had higher protein, fat and ash contents than from other processing methods.

Deshpande et al. (2007) conducted pre milling trials on pigeon pea grain employing soy oil-water and CIRCOT microbial consortium enzyme treatments and studied cooking quality of treated samples. The range of variation of solid dispersion during the cooking was observed as 0.24 to 0.80 g/10g for raw grain, 0.27 to 0.85 g/10g for soy oil treated grain and 0.45 to 1.75 g/10g for consortium treated grains. The optimum cooking time was found to be 38 min for soy oil treated grain, 33 min for microbial consortium treated grain as compared to 43 min for raw untreated grain. Based on the results obtained, the microbial consortium pre milling treatment was observed the most appropriate and promising.

Sethi et al. (2008) studied inter relationship between cooking time and some physico-chemical characteristics in pigeon pea (Cajanus cajan) genotypes and reported that the cooking time of whole pigeon pea pulse ranged between 51 and 63 min. indicating a large variation. However, the cooking time of pigeon pea dhal ranged between 19 to 31 min. Correlation coefficient between grain weight and hydration capacity (0.77), grain volume
and grain weight (-0.86) and that of swelling capacity and swelling index (0.90) were found to be significant at 1 % level.

Sreerama et al. (2009) reported that the xylanase and protease enzyme pre-treatments did not altered the cooking properties of dehulled legumes. These results indicated that partial degradation of non starch polysaccharide (NSP) and/or proteins of mucilage which prevails in between hulls and cotyledon by enzyme had facilitated the improvement in the dehulling properties of legumes.
CHAPTER III
THEORETICAL CONSIDERATION

The literature regarding the origin of enzymes, enzyme classification, physical and chemical properties of enzymes, functions of enzymes, factor affecting enzyme reaction, enzyme kinetics and structure of enzyme has been reviewed. The important theoretical aspects are given below under different sections.

3.1 Enzyme

Enzyme is an important class of globular proteins of biological origin that act as biochemical catalyst and speed up the rate of biochemical reactions without its consumption in the process. The most distinguishing property of an enzyme in its catalytic action is its specificity and selectivity. Each enzyme catalyses only a specific reaction involving a specific substrate and hence it's great value to chemists and engineers. Another major characteristic of enzyme is its sensitivity to the conditions in which it operates. The enzymes are functional only within a specific range of pH, temperature and presence of inhibitors, cofactors, etc. A very useful property of enzymes as catalysts is that they are generally required in very small quantities (Rama Rao, 2002).

3.1.1 Enzyme classification and nomenclature

Based on catalyzed reactions, the nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) recommended the following classification (Rama Rao, 2002).

1. Oxidoreductases: Catalyze a variety of oxidation-reduction reactions. Common names include dehydrogenase, oxidase, reductase and catalase.

2. Transferases: Catalyze transfers of groups (acetyl, methyl, phosphate, etc.). Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.
3. Hydrolases: Catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. Common examples are proteases splits protein molecules and cellulases break down cellulose to beta-glucose. Cellulase acts on cellulose molecules by hydrolysing the b-1, 4 glycosidic linkages.

4. Lyases: Catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. Common names include decarboxylase and aldolase.

5. Isomerases: Catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.

6. Ligases: Catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

The general principles of current classification and nomenclature of enzymes are (i) Enzyme names, especially those ending with -ase, should be used only for single enzymes, i.e., single catalytic entities, (ii) an enzyme is classified and named according to the specific reaction which it catalyses and (iii) enzymes can be divided into groups on the basis of chemical reaction catalysed.

Each enzyme is given a four number code, e.g., EC 3.2.1.8. The first number designates one of the six main divisions, the second number indicates the sub class, the third number indicates the sub- subclass and fourth number is the serial number of the enzyme in its sub-subclass. (Blackstock, 1998)

3.1.2 Physical and chemical properties of enzymes

Xylanase: Xylanase is complex hydrolytic enzyme preparation which has pronounced effect on complicated hemicellulose substrates containing xylan, manan, glucan, etc. Xylanases are genetically single chain glycoproteins, ranging from 6–80 kDa, active between pH 4.5 to 6.5 and temperature between 40 to 60 °C (Butt et al., 2008).

Cellulase: Most cellulases studied have similar pH optima, solubility and amino acid composition. Thermal stability and exact substrate specificity may vary. Generally, cellulase preparations contain other enzymes in addition to
cellulose which may also affect the properties of the preparations. The optimum pH and temperature lies between 4.0 to 5.0 and 40 to 50 °C, respectively. Cellulase is inhibited by its reaction products e.g. glucose, cellobiose. Mercury (Hg) inhibits cellulases completely, whereas Mn, Ag, Cu and Zn ions are only slightly inhibitory (Whitaker, 1971).

**Pectinase**: Pectinase catalyses hydrolysis of pectin into galacturonic acid and pectic acid. Colour of pectinase enzyme is light brown and soluble in water. The optimum pH and temperature range for pectinase enzyme is 3.5 to 4.5 and 40 to 50 °C, respectively (Whitaker, 1971).

### 3.1.3 Function of Enzymes

**Xylanases (EC 3.2.1.8)**: Xylanase degrade the linear polysaccharide beta-1, 4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants. The most important enzyme is endo-1,4-xylanase (EC 3.2.1.8), which initiates the conversion of xylan into xylooligosaccharides. Xylosidase, debranching enzymes (L-arabinofuranosidase and glucuronidase) and esterases (acetyl xylan esterase, feruloyl esterase) allow the complete degradation of the xylooligosaccharides to their monomeric constituents (Whitaker *et al.*, 2003).

**Cellulases (EC 3.2.1.4)**: Cellulases break down cellulose to beta-glucose. Cellulase acts on cellulose molecules by hydrolysing the b-1,4 glycosidic linkages. It largely produces cellobiose, which can ultimately yield glucose units, depending on the characteristic of the enzyme (Whitaker *et al.*, 2003).

**Pectinases (EC 3.3.1.15)**: Pectinase breaks down pectin, a polysaccharide substrate found in the cell wall of plants, into simple sugars and galacturonic acid. Pectinases break down the pectin to pectinic acid and finally pectic acid (Whitaker *et al.*, 2003).

### 3.1.4 Factors affecting enzyme reactions

There are numerous factors affecting the enzyme action and these factors should be considered for the enzyme applications (Rama Rao, 2002).
Concentration of enzyme: For most enzymatic reactions, the rate of the reaction is directly proportional to the concentration of enzyme, at least during the early stage of the reaction.

Concentration of substrate: The rate of enzyme reaction is proportional to the substrate concentration at very low substrate concentration. With an excess amount of substrate, the extent of enzyme reaction shows a linear relationship between the reaction time and the amount of product formed during the early stage of the reaction. As the enzymatic reactions proceed, the amounts of substrate decrease, which causes the reaction to slow down.

pH: pH has a significant influence on its activity due to the protein nature of enzymes. The optimum pH at which enzymes show the highest activity vary widely with the enzyme. Enzyme activities decrease rapidly above or below the optimum pH until the enzymes are completely denatured and inactivated. Also, there is a pH range at which enzymes are most stable, which does not always coincide with the optimum pH.

Time: As mentioned above, the reaction rates slow down during the course of the enzymatic reaction due to many factors such as a reduction in the amount of substrate available or the inhibitive action of end products. Therefore, it is important in enzyme applications that sufficient time be allowed for the enzyme reactions to approach completion.

Temperature: Enzymes are denatured at high temperatures as well as at extremely low temperatures, losing their ability to catalyze reactions. The other effect is an acceleration of the reaction rate at high temperatures. Like most chemical reactions, the rate of an enzyme reaction increases with increasing temperature, up to a certain point, known as the optimum temperature. Moreover, it should be considered that even for a single enzyme reaction, the optimum temperature changes mainly depending upon the substrate concentration or incubation time. Also, the inactivation temperature varies depending upon the particular enzyme.

Moisture content: The moisture content and circumstance in which enzymes are present has a profound effect on enzyme activity. In the presence of
sufficient substrate, lack of moisture can inhibit enzyme reactions because all enzyme reactions occurred in aqueous systems. There are soluble commercial enzymes which are stable in their dry powder state but start to react as soon as the enzymes are exposed to water.

**Activators:** Many molecules and ions, when present in the reaction medium affect the rate of enzyme-catalyzed reactions. These substances bind to the enzyme or the enzyme-substrate complex, thereby, affecting the rate. Substances which increase the rate of reaction are termed as enzyme activators. Activators are very important in the cellular regulation of enzymes. Many enzymes require the presence of an additional, non protein, cofactor. Some of these are metal ions such as Zn$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, K$^+$, and Na$^+$. Some cofactors are small organic molecules called coenzymes. The B vitamins, i.e., thiamine (B1), riboflavin (B2) and nicotinamide are precursors of coenzymes.

A cofactor is a non-protein chemical compound that is bound to a protein and is required for the protein’s biological activity. These proteins are commonly enzymes, and cofactors can be considered “helper molecules” that assist in biochemical transformations. Cofactors are either organic or inorganic. They can also be classified depending on how tightly they bind to an enzyme, with loosely-bound cofactors termed coenzymes and tightly-bound cofactors termed prosthetic groups. Some sources also limit the use of the term “cofactor” to inorganic substances. An inactive enzyme, without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is the holoenzyme.

### 3.1.5 Enzyme terminology

**Enzyme units:** The enzyme unit (U) is a unit for the amount of a particular enzyme. One U is defined as the amount of the enzyme that catalyzes the conversion of 1 micro mole of substrate per minute. The conditions also have to be specified: one usually takes a temperature of 25°C and the pH value and substrate concentration that yield the maximal substrate conversion rate (Hames and Hooper, 2000).

1 enzyme unit (U) = 1µmol/min
**Enzyme activity:** Enzyme activity is a measure of the quantity of active enzyme present. The SI unit is the katal, $1 \text{katal} = 1 \text{ mol/ s}$, but this is an excessively large unit. A more practical and commonly used value is 1 enzyme unit (U) = 1 $\mu$mol/min (Rama Rao, 2002).

Enzyme activity = moles of substrate converted per unit time

= rate of reaction $\times$ reaction volume. ...3.1

**Specific activity:** The specific activity is the activity of an enzyme per milligram of total protein (expressed in $\mu$mol/ min mg). Specific activity gives a measurement of the activity of the enzyme. It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of total protein (Rama Rao, 2002).

\[
\text{Specific activity} = \frac{\text{rate of reaction} \times \text{reaction volume}}{\text{mass of total protein}} \quad \ldots3.2
\]

Specific activity is a measure of enzyme purity. The value becomes larger as an enzyme preparation becomes more pure, since the amount of protein (mg) is typically less, but the rate of reaction stays the same (or may increase due to reduced interference or removal of inhibitors)

**Turnover number:** The number of substrate molecules transformed per minute by a single enzyme molecule is defined as Turnover number. If the molecular weight of the enzyme is known, the turnover number can be calculated from the specific activity (Rama Rao, 2002).

**Rate of a reaction:** Rate of reaction is the concentration of product produced per unit time. ($\text{mol/ L.s}$). The rate of a chemical reaction is affected by the total number of enzymes as well as the concentration of substrates. It can describe the reaction rate with a simple equation to understand how enzymes affect chemical reactions (Rama Rao, 2002).
Purity:

\[
\text{Purity} (\%) = \frac{\text{specific activity of enzyme sample}}{\text{specific activity of pure enzyme}} \quad \ldots 3.3
\]

The impure sample has lower specific activity because some of the mass is not actually enzyme. If the specific activity of 100% pure enzyme is known, then an impure sample will have a lower specific activity, allowing purity to be calculated (Rama Rao, 2002).

Specificity: Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity (Rama Rao, 2002).

3.1.6 Michaelis-Menten saturation curve of an enzyme reaction

In the absence of enzymes, the rate of a reaction can be thought to increase linearly with substrate concentration (Fig.3.1). The reaction rate is given as \( \frac{dp}{dt} \), or the change in product over time (Lehninger, 1982).

\[
\frac{dp}{dt} = S \times k \quad \ldots 3.4
\]

Where, \( S \) is the substrate concentration, and \( k \) is the frequency at which substrate is converted to product.

The rate of a reaction involving enzymes also increases as the substrate concentration increases. However, the number of enzyme active sites available is limited. At low enzyme concentrations or high substrate concentrations, all of the available enzyme active sites could be occupied with substrates. Therefore, increasing the substrate concentration further will not change the rate of diffusion. In other words, there is some maximum reaction rate (\( V_{max} \)) when all enzyme active sites are occupied. The reaction rate will increase with increasing substrate concentration, but must asymptotically approach the saturation rate, \( V_{max} \). \( V_{max} \) is directly proportional to the total enzyme
concentration, $E$, and the catalytic constant of the enzyme, $k_{cat}$, which describes the frequency at which the enzyme-substrate complex is converted to product. How quickly enzyme active sites become saturated can be described by the variable $K$, the substrate concentration at which the reaction rate is $V_{max}$. $K$ is called the Michaelis-Menten constant after the scientists who originally derived it. The reaction rate can be described by the equation

$$\frac{dp}{dt} = \frac{V_{max} \times S}{S + K} = \frac{k_{cat} \times E \times S}{S + K} \quad \ldots 3.5$$

Where, $S$ is the substrate concentration. We can graph $dp/dt$ as a function of $S$ to see how the reaction rate changes with increasing substrate concentration for reactions in the presence and absence of enzymes.

![Graph showing initial velocity versus substrate concentration](image)

**Fig. 3.1 Plot of initial velocity versus substrate concentration**

As we can see, the rate of product formation increases with increasing substrate concentration. However, in the presence of enzymes the function $dp/dt$ has higher values and a much steeper slope. This implies enzymes greatly increase the reaction rate. However, as the enzymes become saturated, the reaction rate levels off.
By plotting $dp/dt$ as a function of $S$ for two different values of $K$ (1 and 2 lines), we can see how the Michaelis-Menten constant affects the reaction. When $K$ is small (line 1 with value $K1$) the reaction approaches $V_{\text{max}}$ much more quickly.

Now we will hold $K$ constant and plot $dp/dt$ as a function of $S$ for different values of $E$.

![Plot of initial velocity versus substrate concentration at constant $K$](image)

**Fig. 3.2 Plot of initial velocity versus substrate concentration at constant $K$**

When the enzyme concentration is small, $V_{\text{max}}$ is much smaller. The reaction rate still increases with increasing substrate concentration, but levels off at a much lower rate. By increasing the enzyme concentration, the maximum reaction rate greatly increases.

The rate of a chemical reaction increases as the substrate concentration increases. Enzymes can greatly speed up the rate of a reaction. However, enzymes become saturated when the substrate concentration is high. Additionally, the reaction rate depends on properties of the enzyme ($K$, $k_{\text{cat}}$) and the enzyme concentration ($E$).

### 3.1.7 Lock and key model

Enzymes are very specific and it is because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. However, while
this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve.

\[ \text{Fig. 3.3 Induced fit hypothesis of enzyme action} \]

Koshland (1958) suggested a modification to the lock and key model. Since, enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site, the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proof reading mechanism.

\subsection{3.1.8 3D structure of enzymes}

Like all proteins, enzymes are long, linear chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a specific structure, which has unique properties. (Fig.3.7)
(i) Xylanase (Source: Uzuner et al., 2010)

(ii) Cellulase (Source: Rabinovich et al., 2002)

(iii) Pectinase (Source: Danielle et al., 2009)
3.1.9 Enzymatic action

Xylanase enzymes digest xylan polymers, which are a major constituent of the hemicellulose, into xylose. Xylans are heteropolymers consisting primarily of the pentose sugars xylose and arabinose. (Fig. 3.4). (Butt et al., 2008)

Fig. 3.4 3D structure of xylanase, cellulase and pectinase

Cellulase enzyme catalyzes the hydrolysis of cellulose into glucose (Figure 3.5). (Xiang et al., 2003)
**Fig. 3.6 Chemical structure of cellulose and point of cellulase action**

Pectinase action: Pectinase breaks down pectin, a polysaccharide substrate found in the cell wall of plants into pectic acid. (Fig. 3.6) (Singh *et al.*, 2005)

![Chemical structure of cellulose and point of cellulase action](image)

The arrow indicates the place where the pectinase reacts with the pectin

**Fig. 3.7 Chemical structure of pectin and point of pectinase action**

**3.1.10 pH and temperature optima of enzymes**

The pH optima for the three activities in the CLEA were more or less same as those compared to free enzyme activities. The pH optima were broad (at 4.5 for pectinase and 5.5 for xylanase and 5 for cellulase activities) and agreed with the literature value (Sreenath, 1993, Gawande and Kampat, 1998, Suh *et al.*, 2002). In the case of cellulase activity, CLEA showed pH optima at 5.5 as compared to 5 observed and reported for free enzyme.

The temperature optima also did not reveal significant changes. The temperature optima observed for free enzymes agreed well with the reported value (Sreenath, 1993, Gawande and Kampat, 1998, Suh *et al.*, 2002). The pectinase temperature optima was 50 °C for both free enzyme and CLEA. Free xylanase showed a temperature optimum of 50 °C. CLEA showed broad temperature optima in the range of 50–55 °C. Free cellulase had a temperature optimum of 50 °C.

The pH optima of pectinase, xylanase and cellulase in CLEA were studied over the pH range of 3.5–9.5. Temperature optima of pectinase, xylanase and cellulase in free form and in CLEA were studied over the range of 25–70 °C.
3.1.11 Mixed activity enzymes

The mixed activity enzyme was reported to be more effective than pure enzymes in improving oil yield (Bhatnagar and Johri, 1987).

The combined treatment of pectolytic and cellulolytic enzymes gave good results in enzymatic fruit juice processing (Bezusov et al., 1989; Traversi et al., 1988). Bezusov et al. (1989) found an optimum ratio of pectolytic and cellulolytic enzymes as 1:1 for maximum juice yield.

Massiot et al. (1992) reported that the hydrolysis of the polysaccharides by pectinase was facilitating by the presence of cellulase which was necessary for complete liquefaction of tissue.
CHAPTER IV
MATERIALS AND METHODS

This chapter deals with the selection of raw materials for the study, procedures followed for determination of physical properties, proximate composition of pigeon pea grains, laboratory scale dehusking machine, procedures followed for oil and enzymatic pre-treatments, details of structural image analysis, determination of protein content and cooking quality of treated dhal.

4.1 Selection of Variety and Procurement

Amongst different varieties of pigeon pea cultivated in Gujarat, the BDN 2 variety is most commonly grown by the farmers throughout the state. Moreover, BDN 2 variety is milled in the pulse mills of Gujarat on large scale for obtaining 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 the Junagadh Agricultural University, Junagadh during the year 2010-11.

4.1.1 Cleaning and grading

The pigeon pea grains were cleaned manually to remove all foreign matters such as dust, dirt, stones, chaff, immature grains, insect eaten and broken grains. The cleaned grains were then graded by manually operated size grader to obtain uniform sized grains (5.27 to 5.38 mm).

4.1.2 Moisture content

The initial moisture content of pigeon pea grain sample was determined by hot air-oven method. One stage procedure was followed for samples with moisture content less than 13 %. The ground samples about 2 to 3 g each were weighed and transferred into 2 to 3 petri dishes, which were covered with lids immediately. The dishes were uncovered and placed in a hot air oven (NV 858/859 of Nova Instruments Pvt. Ltd, Ahmedabad) at a temperature of 130 ± 1°C for 1 h (Sahay and Singh, 1994). Moisture content of sample was
determined based on drop in weight from initial weight of sample by using following formula.

\[
\text{Moisture content (\% w.b.)} = \frac{\text{Initial wt. of sample - Oven dried wt. of sample}}{\text{Initial wt. of sample}} \times 100
\]  

\(4.1\)

4.2 Physical Properties of Pigeon pea

Several characteristics of pigeon pea grain affect the dehulling efficiency. Interaction of pre milling treatments and grain characteristics play an important role in determining the dehulling quality. Selection of pre milling treatment also depends on the characteristics of the grain. Different properties of pigeon pea grains, namely size in terms of length, breath and thickness, sphericity, bulk density, porosity, true density, angle of repose and coefficient of static friction against different surfaces were determined as described in the subsequent sections hereunder.

4.2.1 Sample preparation

The sample of desired moisture levels were prepared by adding the required amount of distilled water as calculated by following equation (Coskun et al., 2005).

\[
Q = \frac{W_i \times (M_f - M_i)}{(100 + M_i)}
\]  

\(4.2\)

Where,

\(Q\) = mass of water to be added (g),
\(W_i\) = initial mass of sample (g),
\(M_i\) = initial moisture content of sample (\% d.b.) and
\(M_f\) = final moisture content of sample (\% d.b.)

The samples mixed thoroughly with calculated amount of water were then filled into separate plastic jar. The samples were kept at 5 °C in a refrigerator (Samsung, Model: RT26ADTS) for a week to enable the moisture to distribute uniformly throughout the sample. Before starting a test, the required quantity of the grains was taken out of the refrigerator and allowed to equilibrium to the
room temperature for about 2 h (Coskun et al., 2005). All the physical and frictional properties of the grains were determined at five levels of moisture contents viz., 10± 0.5, 15± 0.5, 20± 0.5, 25± 0.5 and 30± 0.5% (d.b.) with fifteen replications.

4.2.2 Size and sphericity

The principal dimensions of pigeon pea grains in terms of length, width and thickness were measured with the help of digital vernier caliper (Mityutoyo-Japan, Model: CD-12") with reading accuracy ± 0.01 mm. The longest dimension in the longitudinal direction was considered as length while the smallest dimension was measured as thickness of grain (Fig. 4.1). The axial dimensions were measured for fifteen grains randomly selected from the bulk sample of pigeon pea. The average value of fifteen readings was determined.

![Figure 4.1: Measurement of maximum (a), intermediate (b) and minimum (c) intercepts of pigeon pea grain](image)

The size and sphericity were then calculated using the following formulae (Mohsenin, 1986).

Size = \((a \times b \times c)^{1/3}\)  

Sphericity = \(\frac{(a \times b \times c)^{1/3}}{a}\)

Where,

- \(a\) = length of grain, mm
- \(b\) = width of grain, mm
- \(c\) = thickness of grain, mm
4.2.3 Thousand grain mass

Thousand grain mass was determined by counting 1000 grains and weighing them through a digital electronic balance (Metler, Model: PE 360) having an accuracy of ± 0.001 g.

4.2.4 Bulk density

The bulk density of the pigeon pea grains was determined using the standard test weight procedure (Garnayak et al., 2008) by filling a container of 500 ml with the grains from a height of 150 mm at a constant rate and then weighing the content. No separate manual compaction of grains was done. The bulk density was calculated from the mass of grains divided by the volume of the container and bulk density was expressed in g/cc.

\[ \rho_b = \frac{W}{V} \]  

...(4.5)

Where,

\( W = \) Mass of grains, g

\( V = \) Volume occupied by the same grains, cc

4.2.5 True density

The true density of pigeon pea grain is defined as ratio of the mass of sample to the true volume of the same sample. True density was determined by the liquid displacement method using toluene (Mohsenin, 1986), as it has little tendency to penetrate into the grains. Pigeon pea grains will not absorb toluene within the short time. True density of toluene was 0.87 g/cc. The volume of toluene displaced was found by immersing a weighed quantity of pigeon pea grains in toluene. True density was then calculated using the following formula. The measurement of true density was replicated five times and average was expressed in g/cc.

\[ \rho_t = \frac{W}{V_t} \]  

...(4.6)
Where,

\[ W_t = \text{Mass of grains, g} \]
\[ V_t = \text{True volume occupied by the same grains, cc} \]

### 4.2.6 Porosity

The porosity of pigeon pea grains at selected levels of moisture content was calculated from the values of bulk and true densities using the following equation as described by Mohsenin (1986).

\[
\text{Porosity} = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 \quad \ldots (4.7)
\]

Where,

\[ \rho_b = \text{Bulk density} \]
\[ \rho_t = \text{True density} \]

### 4.2.7 Angle of repose

The angle of repose of pigeon pea grains was determined by standard circular platform method as given by Mohsenin (1986). A box having circular platform (Fig. 4.2) fitted inside was filled with pigeon pea grains. The circular platform was surrounded by a metal funnel leading to a discharge hole. The extra grains surrounding the circular platform were automatically discharged through the funnel leaving a free standing cone of pigeon pea grains on the platform. A stainless steel scale was used to measure the height of cone and angle of repose was calculated by the following equation

\[
\theta = \tan^{-1}\left(\frac{h}{d}\right) \quad \ldots (4.8)
\]

Where,

\[ \theta = \text{Angle of repose} \]
\[ h = \text{Height of cone, cm} \]
\[ d = \text{Diameter of cone, cm} \]

### 4.2.8 Static coefficient of friction

The static coefficient of friction of pigeon pea grains was determined considering three different surfaces, namely glass, galvanized sheet and plywood surface. The coefficient of friction was determined by the inclined plane (Fig. 4.3) method as described by Mohsenin (1986). The grains were
placed on the test surface at the top edge in the container without bottom. The inclined plain was tilted till the container filled with grain starts moving over the inclined surface. The vertical (Y) and horizontal (X) distances were measured with help of scale. The values of Y and X were used to calculate the coefficient of friction using the following equation.

Coefficient of friction, \( \tan \Phi = \frac{Y}{X} \) ....(4.9)

Where,

Y = Vertical distance, cm
X = Horizontal distance, cm

Fig. 4.2 Apparatus for measurement of angle of repose

Fig. 4.3 Tilting table apparatus for measurement of coefficient of friction
4.2.9 Statistical analysis
The statistical analysis of the experimental data on different physical properties at five levels of moisture content was carried out using completely randomized design and analysis of regression (curve fitting) was carried out using Microsoft Excel Software.

4.3 Proximate Composition of Pigeon pea Grain
The proximate compositions of pigeon pea grains, viz., carbohydrate, protein, fat, crude fibre and ash content were determined at 10.46 %, (w.b.) moisture content as described in the subsequent sections hereunder.

4.3.1 Carbohydrate
The carbohydrate content was determined according to the method reported by Sadasivam and Manickam (1992). A 100 g sample in a boiling tube was hydrolyzed by keeping it in boiling water bath for three hours with 5 ml of 2.5N hydrochloric acid and cooled to room temperature. Then it was neutralized with solid sodium carbonate until the effervescence ceases. Supernatant was collected and 0.5 and 1 ml aliquots taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard (10 ml of stock solution into 100 ml distilled water). ‘0’ served as blank. The volume was made up to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent (200 mg anthrone dissolved in 100 ml of ice cold 95 % H$_2$SO$_4$) was added and heated for 8 min in a boiling water bath. It was cooled rapidly and green to dark green colour was read at 630 nm on spectrophotometer (Systronics Model: UV-VTS 108). A standard graph was drawn by plotting concentration of the standard on the x-axis versus absorbance on the y-axis. Amount of carbohydrate present in the sample tube was determined using the standard graph. Amount of carbohydrate present in 100 mg of sample was calculated by the following formula

\[
\text{Carbohydrate, \%} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100 \quad \text{...(4.10)}
\]
4.3.2 Protein

The protein content of enzymatic and oil treated dhal was determined by NIR spectroscopy method. Instalab 600 NIR product analyzer made by Dickey-john Corporation (Plate 4.1) was used for the determination of oil and protein content of pigeon pea dhal obtained from different pre-treatments.

Near Infrared (NIR) radiation lies in the spectral region between 750-2500 nm in the electromagnetic spectrum, i.e., between the visible and mid-infrared. Near infrared spectroscopy is based on molecular overtone and combination vibrations. Such transitions are forbidden by the selection rules of quantum mechanics. As a result, the molar absorptive in the near Infrared region is typically quite small. One advantage is that, NIR can typically penetrate much deeper into a sample than mid infrared radiation.

After switching on the analyzer, it was allowed to warm up for 10 to 15 minutes. Thereafter the key “2” was pressed (which was for pigeon pea grain powder) and then the key “prod” was pressed. When screen displayed “INSERT SAMPLE”, ground samples of dhal were inserted in the analyzer. The numeric keys entered numerical data into the computing circuits and initiates selected mode sequences. Therefore, after pressing the “Step” key, the digits 2.2 was pressed on keyboard for obtaining the protein content on screen whereas the digits 2.3 was employed for getting oil content of pigeon pea dhal.

4.3.3 Oil

The oil content in pigeon pea grain was estimated by soxhlet extraction method. 5 g of pigeon pea grains were extracted for 8 h with petroleum ether at 60–80 °C as per the method given by AOAC (2005). The solvent was distilled out and the flasks were then transferred to oven maintained at 80 °C for 24 h. The flasks were removed from oven and kept in desiccator until its temperature equilibrates room temperature. The flasks were then weighed and per cent oil was calculated as,

\[
\text{Oil, } \% = \frac{\text{Weight of flask + Oil - weight of flask}}{\text{Weight of sample (g)}} \quad \text{(4.11)}
\]
Plate 4.1 NIR spectroscopy for protein measurement

Plate 4.2 Fibertherm for measurement of fibre content
4.3.4 Crude fibre

Fibretherm Gerhardth plus instrument (make: Pelican Equipments, Chennai) was used for estimation of crude fibre content (plate 4.2). Pigeon pea dhal sample was treated with 1.25 % H$_2$SO$_4$ followed by 1.25 % NaOH and washed thoroughly with distilled water after each treatment. Neutral residue left over was dried, weighed and then ignited into muffle furnace. From the loss in weight of the residue, the percentage of crude fibre was calculated using following formula given by Ranganna (1986).

\[
\text{Crude Fibre, } \% = \frac{W_2 - W_3}{W_1} \times 100
\]

Where,

- $W_2 =$ Weight of digested sample, g
- $W_3 =$ weight of ash, g
- $W_1 =$ Initial sample weight, g

4.3.5 Ash

The ash content was determined according to the method referred by AOAC (2005). 5 g of the pigeon pea grain sample was taken into a silica dish. The dish with content was ignited on a bunsel burner. The material was then ashed at 798 °K for 4 h in a muffle furnace. The dish was cooled and weighed. The total ash content was calculated by difference in weights and was expressed as per cent ash.

4.4 Dehusking Machine

The laboratory scale dehusking machine (for dhal milling) fabricated by Bharodia (2004) with overall dimensions of 600 mm x 620 mm x 935 mm, capacity 85 kg/h, power unit 1.0 hp electric motor was used for all the milling studies.

4.4.1 Construction

The dehusking machine mainly consisted of an emery roller mounted on a shaft. The different components of the machine along with their dimension are given in Fig.4.4. The diameter of emery roller was 190 mm,
Fig. 4.4 Laboratory scale dehusking machine
which included the coating of emery (grade No. 22, as adopted by the pulse millers). A slotted screen (3 mm circular opening) was mounted concentrically around the emery roller cylinder. The inner diameter of slotted screen was 210 mm. The screen was made in two equal halves to facilitate cleaning. These two halves were held in position through clamps and latches. A provision was also made for adjusting the clearance between the screen and the emery roller either at top or bottom sides. The clearance of 10 mm between emery roller and slotted screen was fixed for present study based on physical dimensions of grain. The inclination was provided by keeping the inlet side about 25 mm higher than the outlet side for smooth flow of grains from inlet to outlet (Singh, 1999). The shape and volume of the hopper were trapezium and 2767.5 cm$^3$, respectively. The inlet feed control mechanism with 50 x50 mm square opening was provided to control the flow rate of grains in the machine. The feed rate could be controlled by moving sliding gate. The outlet control mechanism 50 x 30 mm opening with sliding gate was also provided to the finished product outlet in order to control the retention time of grains in the machine. The powder collecting trough was made from ordinary transparent polyethylene sheet available in the market and was fixed with frame by use of Velcro. The Velcro arrangement facilitated easy fixing and removal of polyethylene trough (powder collecting trough) required for removing the slotted screen for cleaning purpose. The single phase electric motor of 1.0 hp was provided to operate dehusking machine. Plate 4.3 shows the developed laboratory scale dehusking machine while Plate 4.4 shows the components of the machine.

4.4.2 Working principle

Dehulling of pretreated and conditioned grains is generally done using emery rollers. The emery rollers are abrasive rolls made up of carborundum and encaged in affixed perforated cylindrical screen. Grains enter into the space between the abrasive roll and perforated shell, take considerable period to travel from one end to another. During this time, the grains are subjected to a combination of impact and frictional forces (Saxena, 1985).
Plate 4.3 Laboratory scale dehusking machine

Plate 4.4 Components of laboratory scale dehusking machine
Pigeon pea grains are first of all comes in contact with emery roller which is rotating around its axis. Once the grain comes in contact of abrasion made over the roller, the circular motion of roller creates a centrifugal action by which the grains thrown towards the concave resulting into the impact. Therefore, the centrifugal as well as impact forces are also playing the role in dehusking besides the abrasion. As the speed of roller increases, the impact force also increases and attains some limiting value required to dehusk the grain.

4.4.3 Sample size
Bharodia (2004) worked out the minimum size of sample (2 kg) required to conduct the milling tests on the basis of dehusking machine dimension and was considered for milling tests of enzymatic and control samples of pigeon pea grains.

4.4.4 Operating parameters
All the tests were conducted at standard settings of the dehusking machine as reported by Bharodia (2004) and also the parameters at which the good experimental results were obtained during the preliminary experiments. The optimum operating speed and feed rate of the dehusking machine were 1420 rpm and 64 kg/h, respectively.

4.5 Dry Milling Method Followed as Control
Generally, the dry 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 considered as control. The cleaned and size graded grains were pitted through dehusking roller machines. Thereafter, mustard oil was used for oil treatment and applied at 0.5 kg oil per 100 kg pigeon pea grains (Patel et al., 2000). For 2 kg pigeon pea grains 10 g mustard oil was mixed and kept in a glass bottle for 36 h for diffusion of oil. After 36 h, the water was sprayed (100g/2 kg grain) on the grain and heaped for 12 h. Subsequently after tempering, the grains were dried in a mechanical dryer (tray dryer) at 60°C up to a moisture content of 10±0.5 % (w.b.). This sequence of operation was repeated three to four times. The samples were than milled in a dehusking machine at optimum operating conditions. During each dehusking operation, husk, powder and broken were separated from dehusked split pulse (dhal) for the analysis of hulling efficiency. Dehusked splits obtained in this
operation were considered as ‘second grade’ because their edges were not sharp and usually rounded-off by scouring. The process flow chart followed during the experiment of milling of pigeon pea grains is given in Fig. 4.5

Raw pigeon pea
↓
Cleaning
↓
Grading (2 kg)
↓
Pitting (Emery roller)
↓
Pre-treatment with mustard oil @ 0.5 %
(10 g / 2 kg)
↓
Tempering (36 h)
↓
Addition of water @ 5 %
(100 g / 2 kg)
↓
Drying (Tray dryer)
(60 °C, 10 ± 0.5 % (w.b.) Moisture content)
↓
Dehusking and splitting
↓
Separation→Broken, husk, unhulled grains,
powder
↓
Dhal

Fig. 4.5 Flow chart for dry milling method of pigeon pea grains

4.6 Selection of Enzymes

Enzyme is a biochemical catalyst and speed up the rate of biochemical reactions without its consumption in the process. The most distinguishing property of an enzyme in its catalytic action is its specificity and selectivity. Each enzyme is catalyzed only a specific reaction involving a specific substrate. Therefore, the selection of enzymes was based on the chemical composition and binding substances present between husk and cotyledon of pigeon pea
grain. The xylanase enzyme being widely used as biobleaching agent for lignin isolation (Browning, 1967). Cellulase and pectinase break down cellulose to beta-glucose and pectin to pectinic acid and finally pectic acid. The xylanase, cellulase and pectinase are the key enzymes that rupture the binding materials leading to increase the dehulling efficiency. Hence, commercial food grade enzymes selected for enzymatic treatment were obtained from their manufacturers. The Xylanase was procured from Advanced Enzyme Technologies Ltd., Thane (Maharashtra) while Cellulase and Pectinase enzymes were obtained from HiMedia Laboratories Pvt. Limited, Mumbai (Maharashtra) as shown in Plate 4.5 and 4.6. The enzyme is very sensitive to the conditions in which it operates. Therefore, it is essential to follow the range of various characteristics specified by their manufacturer as given in Table 4.1.

Table 4.1 Summery of commercial enzyme characteristics supplied by the manufacturer

<table>
<thead>
<tr>
<th>Characteristics</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</td>
<td>2-8 °C</td>
<td>2-8 °C</td>
<td>2-8 °C</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>

4.6.1 Standardization of ratio of enzymes

Preliminary trials were undertaken to arrive at standard ratio of enzymes, i.e., xylanase : pectinase : cellulase. Initially, the proportion of all the three enzymes was selected arbitrarily as given in Table 4.2. The effect of selected enzyme combination on husk removal of pigeon pea grain was evaluated keeping the enzyme concentration, incubation time, incubation temperature, pH and moisture content constant based on the
Plate 4.5 Enzymes in aluminum foil and plastic bottle packages

Plate 4.6 Enzymes in fine powder form
manufacturer’s recommendation (Table 4.1). Based on the results obtained, 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. Again, the proportion of the enzyme combination giving the best result was varied as 3 : 1 : 1 (Xylanase: Pectinase: Cellulase- 60: 20: 20 %) for evaluating the effect of maximum proportion of xylanase on the husk removal and hulling efficiency.

Table 4.2 Different proportion of enzymes

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Enzyme combination (Xylanase : Pectinase : Cellulase)</th>
<th>Enzyme concentration (mg/100g dry matter)</th>
<th>Incubation time (h)</th>
<th>Incubation temperature (°C)</th>
<th>Tempering water pH</th>
<th>Moisture content % (w.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 1 : 1 (33.33: 33.33: 33.33%)</td>
<td>40</td>
<td>9</td>
<td>50</td>
<td>5.0</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>2 : 1 : 1 (50.00: 25.00: 25.00%)</td>
<td>40</td>
<td>9</td>
<td>50</td>
<td>5.0</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>1 : 2 : 1 (25.00: 50.00: 25.00%)</td>
<td>40</td>
<td>9</td>
<td>50</td>
<td>5.0</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>1 : 1 : 2 (25.00: 25.00: 50.00%)</td>
<td>40</td>
<td>9</td>
<td>50</td>
<td>5.0</td>
<td>26</td>
</tr>
</tbody>
</table>

### 4.6.2 Enzymatic pre-treatment

The enzyme solution was prepared at the standardized proportion of all the three selected enzymes. The process flowchart of enzymatic pre-treatment is given in Fig.4.6 for milling of pigeon pea. In case of enzymatic pre-treatment, the degumming may be due to the action of different enzymes used for pre-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.
Distilled water (420.0 ml) ↓ Raw pigeon pea ↓ Cleaning ↓ Grading (2 kg) ↓ Additions of enzyme (358.16 to 1074.48 mg for concentration of 20 to 60 mg/100 g dry matter) ↓ Enzyme solution ↓ Addition of enzyme solution (420.0 ml) ↓ Moisture equilibrium 26.0 % (w.b.) m.c. ↓ Enzyme concentration (20 to 60 mg/100 g dry matter) ↓ Enzyme treated sample ↓ Incubation for hydrolysis (Temperature 40 to 60 °C, Incubation time 3 to 15 h) ↓ Drying (Tray dryer) (60 °C, 10 ± 0.5 % (d.b.) m.c.) ↓ Dehusking and splitting (using Dhal mill) ↓ Separation (Broken, husk, unhulled grains, powder) ↓ Dhal

**Fig. 4.6 Flow chart for enzymatic pre-treatment and milling of pigeon pea**

**pH adjustment:** The distilled water was mixed with 37% hydrochloric acid to adjust pH 4 to 6 with an interval of 0.5 pH (Yoo, 2007). Digital pH meter (Auto pH system PM-300) was used to adjust required pH range (Plate 4.7)
Preparation of enzyme solution: The required amount of pH water solution was calculated using the formula 4.13 and required amount of enzymes were weighed (Plate 4.8) and dissolved in pH water solution. The enzymes were dissolved completely before adding to the pigeon pea grains. The procedure for calculation of weight of the three enzymes required for preparation of different enzyme concentrations are given in Appendix- A.

\[
\text{Weight of water added (g)} = \frac{W_1(M_i - M_f)}{(100 - M_f)} 
\]

Where,
- \( W_1 \) = Initial weight of sample (g)
- \( M_i \) = Initial moisture content, % (w.b.)
- \( M_f \) = Final moisture content, % (w.b.)

For each experiment, 2 kg grains was taken in a stoppered glass bottle and calculated amount of enzyme solution was mixed to increase the initial moisture content from 10.46 % (w.b.) to the desired level moisture content 26.0 % (w.b.). Glass bottle after putting its stopper was shaken with enzyme solution for 3 minutes for equilibrium moisture. The samples were incubated in a humidity oven (Electroquip, Ahmedabad) (Plate 4.9) at 40-60 ± 0.5 \( ^\circ \)C and incubation time (3-15 h). After incubation, the samples were dried in a tray dryer (Plate 4.10) at 60 \( ^\circ \)C to inactive enzyme and to reduce the moisture content to 10 ± 0.5 % (w.b.). Enzyme treated samples in thin layer (25 mm) were spread on a wire mesh tray.
Plate 4.7 pH adjustment using pH meter

Plate 4.8 Weighing of enzymes using digital balance
Plate 4.9 Enzyme treated pigeon pea grains for incubation in humidity oven

Plate 4.10 Drying of samples after enzyme treatment in tray dryer
4.7 Scanning Electron Microscopy

In the Scanning electron microscopy, the specimen was scanned with a focused beam of electrons which produce “secondary” electrons as the beam hits the specimen. These were detected and converted into an image on a television screen, and a three-dimensional image of the surface of the specimen was produced. Microstructure of all the enzymatically hydrolyzed as well as oil treated (control) samples were examined using a Scanning Electron Microscope (EVO 18 Carlzeiss SMT Ltd, Cambridge, England) (Plate 4.11, 4.12)

4.7.1 Sample preparation

Scanning electron microscope utilizes vacuum conditions and use electron to form an image. The specimen samples were prepared by fracturing with the help of forceps and cutter under a light microscope. All water must be removed from the samples because the water would vaporize in the vacuum. All samples need to be made conductive by covering the sample with thin layer of conductive material. The samples were sputter coated with a gold palladium alloy (200 Å thick) for uniform coating. This coating is critical for emission of secondary electrons in order to obtain a quality image having better contrast. Photographs at various magnifications were obtained using 32 mm black and white film (Srivastava et al., 2004).

4.8 Milling of Sample

Enzymatic pre-treated and control samples of 2 kg weight having about 10 ± 0.5 % moisture content (w.b.) were milled using laboratory dehusking machine/dhal mill (Fig. 4.3). Samples were milled at the standard settings of the machine, i.e., 1420 rpm operating speed and 64 kg/h feed rate. After milling, all the 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.
Plate 4.11 Scanning electron microscope showing cavity between seed coat and cotyledon

Plate 4.12 Arrangement of cut samples in sample holder of scanning electron microscope
4.8.1 Dehulled sample separation

The different fractions of the milled product were separated by suitable sieves and hand picking such as whole dehulled grains, split dehulled grains, partly dehulled and unhulled grains, broken, husk and powder (Plate 4.13). A grain was considered completely dehulled when there was no husk adhering to it.

4.8.2 Husk content

The husk (seed coat) content in whole grain was determined by soaking approximately 2 g of pigeon pea grain in distilled water (2 h at 50 °C). The seed coats were then separated manually from the cotyledons, dried in a hot air oven at 100 ± 5 °C up to initial moisture content of 10.46 % (w.b.) (Bharodia, 2004). The data regarding husk content are given in Appendix B. The husk content in % was calculated using the formula 4.14.

\[
\text{Husk content, } % = \frac{\text{Wt. of husk}}{\text{Wt. of pigeon pea grain}} \times 100 \quad \ldots (4.14)
\]

4.8.3 Dehulled fractions

All the fractions were weighed accurately using digital weighing balance with an accuracy of ± 0.01g. (Mettler, model PE 3600). Following equations were used to calculate dehulled fractions obtained by dehulling treatments (Singh et al., 2004)

\[
\text{Husk removed (HR), } % = \frac{\text{HRd}}{\text{Ht}} \times 100 \quad \ldots (4.15)
\]

\[
\text{Coefficient of hulling (Ch)} = 1 - \frac{\text{Wuh}}{\text{Wth}} \quad \ldots (4.16)
\]

\[
\text{Coefficient of wholeness of kernel (Cwk)} = \frac{\text{Wfp}}{\text{Wfp} + \text{Wbr} + \text{Wpo}} \quad \ldots (4.17)
\]

Where,

HRd = Husk removed during dehusking, (g)

Ht = Total husk content (g)

Wfh = husk content in fraction X weight of grain used for milling (g)
Wuh = Weight of unhulled grain after milling (g)
Wth = Weight of grain used for milling (g)
Wfp = Weight of finished product (g) (Splits and whole dehulled grain)
Wbr = Weight of brokens (g)
Wpo = Weight of powder (g)

The hulling efficiency was determined using eq. 4.18

Hulling efficiency (HE) = Ch X Cwk X 100 ...(4.18)

4.9 Cooking Time

Pigeon pea dhal samples obtained through various enzymatic treatments and dry milling method (control) 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 (Plate 4.14). During boiling, the level of water was maintained by regular addition of boiling water. Boiling was continued and samples were drawn at 1 min interval to check the level of cooking by pressing between the thumb and the forefinger till no hard core is left (Plate 4.14) as described by Singh et al. (1984), Full cooking time was recorded as the time when 90% of the dhal were soft enough to masticate (Williams and Singh, 1987)

4.10 Sensory Evaluation

Sensory evaluation was carried out of the cooked samples of enzyme treated and control samples immediately after cooking (Plate 5.4). The cooking was performed in open pot at 98 ± 1.5 °C for 20 min with dhal to distilled water ratio of 1:5. The coded cooked samples were presented to the panelists. The samples were evaluated by ten untrained panelists comprising staff members of different departments of College of Agricultural engineering and Technology. They were asked to rate the samples by six sensory attributes namely, colour, appearance, flavour, texture, taste and overall acceptability on a 9-point hedonic scale of 9 (like extremely) to 1 (dislike extremely) (Meilgaard et al., 1999).
Plate 4.13 Different fractions of pigeon pea grains

Plate 4.14 Samples drawn for cooking test
4.11 Experimental Design

The effects of four independent variables viz., enzyme concentration, incubation time, incubation temperature and tempering water pH on hulling efficiency, protein content and 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. Four response variables, viz., husk removed, hulling efficiency, protein content and cooking time were 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 were used (Khuri and Cornell, 1987). 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 4.3 and 4.4.

4.11.1 Treatment details

1. Enzyme concentration ($X_1$): Five levels, viz.,
   20, 30, 40, 50, 60 mg/100g dry sample
2. Incubation time ($X_2$): Five levels, viz.,
   3, 6, 9, 12 and 15 h
3. Incubation temperature ($X_3$): Five levels, viz.,
   40, 45, 50, 55 and 60 °C
4. Tempering water pH ($X_4$): Five levels, viz.,
   4.0, 4.5, 5.0, 5.5 and 6.0

Total number of treatment combinations

\[ = (2)^{\text{No. of variables}} + (2 \times \text{No. of variables}) + \text{centre points} \quad \ldots (4.19) \]

No of variables: 4

Total number of treatment combinations \( = 2^4 + (2 \times 4) + 6 \)

\[ = 16 + 8 + 6 + \text{Control} = 31 \]
Five different levels for each treatment combinations in coded form were:

- \( \alpha \), -1, 0, +1, +\( \alpha \)

Where, \( \alpha = 2^{\frac{\text{No. of variables}}{4}} = 2^{\frac{4}{4}} = 2 \)

The relationship between the coded and actual values of a factor is given by

\[
\text{Coded value} = X_i = \frac{2(X_i - X_l)}{R_i} \quad \ldots (4.20)
\]

Where,

- \( X_i \) = Actual setting in the uncoded units of the \( i^{th} \) factor
- \( X_l \) = Average of low and high settings for the \( i^{th} \) factor
- \( R_i \) = Range between the low and high settings.

**Table 4.3 Coded and uncoded variables levels**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coded variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
</tr>
<tr>
<td>Enzyme concentration, mg/ 100 g dry matter</td>
<td>(X_1)</td>
</tr>
<tr>
<td>Incubation time, h</td>
<td>(X_2)</td>
</tr>
<tr>
<td>Incubation temperature, (^\circ)C</td>
<td>(X_3)</td>
</tr>
<tr>
<td>Tempering water pH</td>
<td>(X_4)</td>
</tr>
</tbody>
</table>

### 4.11.2 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 (Stat-ease, 2009). Analysis of variance (ANOVA) was conducted for fitting the model represented by Eq. 4.21 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 co-efficient of variation (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

**Table 4.4 Different treatment combinations of variables**

<table>
<thead>
<tr>
<th>Treat. No.</th>
<th>Coded variables</th>
<th>Uncoded (Real) variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>8</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>12</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>16</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>-2</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
optimization using response surface methodology is a second order polynomial equation (Bas and Boyaci, 2007). The model is of the form:

\[ Y_k = b_{k0} + \sum_{i=1}^{3} b_{ki}X_i + \sum_{i=1}^{3} b_{kii}X_i^2 + \sum_{i=1, j=1}^{3} b_{kij}X_iX_j \]

(k=0, 1, 2, 3, …)

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

4.1.1.3 Validity test

The optimum conditions obtained through statistical analysis was verified by conducting the experiment in triplicates. The average value of dehulling efficiency, protein content and cooking time were considered for the validation.
CHAPTER V

RESULTS AND DISCUSSION

This chapter deals with the results obtained from various experiments conducted during investigation which are reported under different sub sections as physical properties and proximate composition of pigeon pea grains (Variety BDN 2), microstructure analysis after enzymatic treatment, hulling efficiency, protein content and cooking time of enzyme treated pigeon pea grains. The data obtained were statistically analyzed and the results are discussed and interpreted for certain conclusions.

5.1 Physical Properties of Pigeon pea Grain

The initial moisture content of the pigeon pea grains was found to be 11.68 % (d.b.). The four other moisture levels obtained after conditioning the grains were 15±0.5, 20±0.5, 25±0.5 and 30±0.5 % (d.b.). The experiments were carried out at the above levels of moisture contents to determine the effect of moisture content on physical properties of pigeon pea grains which are described hereunder. The raw data of derived physical properties of pigeon pea grains at selected moisture content are given in Appendix C(1-2).

5.1.1 Effect on size of pigeon pea grain

The average of three principle dimensions of pigeon pea grains at different moisture contents are presented in Table 5.1. It can be seen that upon moisture absorption, the pigeon pea grain expands in length, width and thickness as the moisture range increased from 10 to 30 % (d.b.). In case of pigeon pea grain of BDN 2 variety, the average length, width, thickness and size of grains increased from 6.05 to 6.32 mm, 5.43 to 5.63 mm, 4.64 to 4.71 mm and 5.337 to 5.510 mm, respectively. The analysis of variance showed that the difference among moisture levels were statistically significant at 5 % level of significance for the length and size while width and thickness were found non significant. Equation 5.1 gives relationship between size and moisture content of the grain while coefficient of determination ($R^2$) value 0.9995 is shown in Fig.
5.1. This could be important consideration in the theoretical consideration of the grain volume at different moisture content. A similar increasing in size was reported by Singh and Kotwaliwale (2010) for physical property of pigeon pea grains.

\[ S = -2 \times 10^{-4} M^2 + 0.0185M + 5.1766, (R^2 = 0.9995) \quad \ldots \quad (5.1) \]

Where,

\[ S = \text{Size, mm} \]

\[ M = \text{Moisture content, \% (d.b.)} \]

Table 5.1 Effect of moisture contents on principle dimensions, size, sphericity and thousand grain mass of pigeon pea grain

<table>
<thead>
<tr>
<th>Moisture Content (% d.b.)</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>Size (mm)</th>
<th>Sphericity</th>
<th>Thousand grain mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6.05</td>
<td>5.43</td>
<td>4.64</td>
<td>5.337</td>
<td>0.883</td>
<td>97.90</td>
</tr>
<tr>
<td>15</td>
<td>6.15</td>
<td>5.49</td>
<td>4.66</td>
<td>5.397</td>
<td>0.877</td>
<td>104.27</td>
</tr>
<tr>
<td>20</td>
<td>6.24</td>
<td>5.56</td>
<td>4.68</td>
<td>5.450</td>
<td>0.874</td>
<td>110.25</td>
</tr>
<tr>
<td>25</td>
<td>6.29</td>
<td>5.59</td>
<td>4.69</td>
<td>5.483</td>
<td>0.872</td>
<td>113.65</td>
</tr>
<tr>
<td>30</td>
<td>6.32</td>
<td>5.63</td>
<td>4.71</td>
<td>5.510</td>
<td>0.871</td>
<td>116.83</td>
</tr>
<tr>
<td>S Em</td>
<td>0.04</td>
<td>0.07</td>
<td>0.06</td>
<td>0.026</td>
<td>0.006</td>
<td>0.60</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>0.13</td>
<td>NS</td>
<td>NS</td>
<td>0.081</td>
<td>NS</td>
<td>1.88</td>
</tr>
<tr>
<td>CV%</td>
<td>1.13</td>
<td>2.21</td>
<td>2.40</td>
<td>0.823</td>
<td>1.248</td>
<td>0.95</td>
</tr>
</tbody>
</table>
5.1.2 Effect on sphericity of pigeon pea grain

The values of sphericity were calculated using Eq. (4.4) and the data on size and the major axis of the grain. The results obtained are presented in Table 5.1. The variation of sphericity with moisture content of pigeon pea grains is shown in Fig 5.2. The sphericity was found to decrease from 0.883 to 0.871 with the increase in moisture content from 10 to 30 % (d.b.). The relationship between sphericity ($S_{ph}$) and moisture content ($M$) with the best fit ($R^2 = 0.9951$) is expressed in Eq. 5.2. The sphericity decreased with increase in moisture content. This probably be due to higher rate of expansion in length compared to breadth and thickness. A similar decreasing in sphericity was reported by Baryeh and Mangope (2003) for pigeon pea and Singh et al. (2008) for chick pea.

$$S_{ph} = 3 \times 10^{-5} M^2 - 0.00184 M + 0.89800, \quad (R^2 = 0.9951) \quad \ldots (5.2)$$

Where,

- $S_{ph} =$ Sphericity
- $M =$ Moisture content, % (d.b.)
5.1.3 Effect on thousand grain mass of pigeon pea grain

The average of thousand grain mass of pigeon pea at different moisture contents are presented in Table 5.1. The thousand grain mass of pigeon pea grain increased from 97.90 to 116.83 g with the increase in moisture content from 10 to 30 % (d.b.). The Analysis of variance showed that the difference among moisture levels were statistically significant at 5 % level of significance for thousand grain mass. The variation of thousand grain mass with moisture content of pigeon pea is shown in Fig 5.3. The relationship between the thousand grains mass (T\textsubscript{1000}) and moisture content (M) was found to be parabolic and can be represented by Eq. 5.3 with $R^2$ as 0.9987. A similar increasing in thousand grains mass has been reported by Baryeh and Mangope (2003) for pigeon pea and Chowdhury et al. (2001) for gram.

$$T_{1000} = -0.0256M^2 + 1.9688M + 80.7240, \quad (R^2 = 0.9987) \quad \ldots(5.3)$$

Where,

- $T_{1000}$ = Thousand grain mass, g
- $M$ = Moisture content, % (d.b.)
Fig. 5.3 Effect of moisture content on thousand grain mass of pigeon pea grains

5.1.4 Effect on bulk density of pigeon pea grain

The experimental results on the bulk density for pigeon pea grains at different moisture contents are given in Table 5.2. The bulk density varied from 872 to 814 kg/m³ and indicated decrease with increase in moisture content from 10 to 30% d.b. (Fig 5.4). The Analysis of variance showed that the difference among moisture contents were statistically significant at 5 % level of significance for the bulk density. This is due to the fact that the increase in mass owing to moisture gain in the grain sample was lower than the corresponding volumetric expansion of the bulk as reported by Zareiforosh et al., 2009. The pigeon pea grain bulk density ($\rho_b$) had the following negative relationship with moisture content (M) as expressed in Eq. (5.4) with $R^2 = 0.996$. A similar decreasing in bulk density was reported by Singh and Kotwaliwale (2010) for pigeon pea and Baryeh and Mangope (2003) for QP-38 variety of pigeon pea.

$$\rho_b = 0.068M^2 - 5.782M + 925, \quad (R^2 = 0.996) \quad .... (5.4)$$

Where,

$\rho_b = $ Bulk density, kg/m³
**Table 5.2** Effect of moisture contents on bulk density, true density, porosity, angle of repose and coefficient of friction of pigeon pea grains (n=3)

<table>
<thead>
<tr>
<th>Moisture Content (% d.b.)</th>
<th>Bulk Density (kg/m³)</th>
<th>True Density (kg/m³)</th>
<th>Porosity (%)</th>
<th>Angle of Repose (degree)</th>
<th>Coefficient of friction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Galvanized surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plywood Surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glass Surface</td>
</tr>
<tr>
<td>10</td>
<td>872</td>
<td>1353</td>
<td>35.47</td>
<td>28.17</td>
<td>0.34</td>
</tr>
<tr>
<td>15</td>
<td>856</td>
<td>1345</td>
<td>36.36</td>
<td>29.21</td>
<td>0.40</td>
</tr>
<tr>
<td>20</td>
<td>836</td>
<td>1331</td>
<td>37.22</td>
<td>30.48</td>
<td>0.44</td>
</tr>
<tr>
<td>25</td>
<td>822</td>
<td>1320</td>
<td>37.73</td>
<td>32.28</td>
<td>0.48</td>
</tr>
<tr>
<td>30</td>
<td>814</td>
<td>1307</td>
<td>37.96</td>
<td>34.08</td>
<td>0.52</td>
</tr>
<tr>
<td>S Em</td>
<td>2.948</td>
<td>3.907</td>
<td>0.23</td>
<td>0.46</td>
<td>0.03</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>9.288</td>
<td>12.311</td>
<td>0.73</td>
<td>1.46</td>
<td>0.08</td>
</tr>
<tr>
<td>CV%</td>
<td>0.608</td>
<td>0.508</td>
<td>1.09</td>
<td>2.61</td>
<td>10.63</td>
</tr>
</tbody>
</table>

![Graph of Bulk Density vs. Moisture Content]

**Fig. 5.4** Effect of moisture content on bulk density of pigeon pea grains

5.1.5 Effect on true density of pigeon pea grain
The variation in true density of pigeon pea grains with moisture content are given in Table 5.2. The true density decreased from 1353 to 1307 kg/m$^3$ with increasing moisture content from 10 to 30 % (d.b.). The effect of moisture content on true density of pigeon pea grains found statistically significant at 5 % level of significance for the true density. The decrease in true density values with the increase in moisture content might be attributed to the relatively higher true volume as compared to the corresponding mass of the grain attained due to the adsorption of water. The variation of true density with moisture content for pigeon pea grains is shown in Fig 5.5. A regression equation with the best fit to the data with $R^2 = 0.996$ is expressed in Eq. 5.5. A similar decreasing in true density was reported by Singh and Kotwaliwale (2010) for pigeon pea and Tavkoli et al. (2009) for soybean.

$$\rho_t = -0.02M^2 - 1.54M + 1371, \quad (R^2 = 0.996) \quad \cdots (5.5)$$

Where,

$\rho_t$ = True density, kg/m$^3$

$M$ = Moisture content, % (d.b.)

![Fig. 5.5 Effect of moisture content on true density of pigeon pea grains](image-url)

5.1.6 Effect on porosity of pigeon pea grain
The values of porosity were calculated using the data on bulk and true densities of pigeon pea grains by using Eq. 4.7 and the results are presented in Table 5.2. The porosity of pigeon pea grains increased from 35.47 to 37.96 % with increasing moisture content from 10 to 30 % (d.b.). The analysis of variance showed that the difference among moisture levels were statistically significant at 5 % level of significance for the porosity. The variation of porosity with moisture content of pigeon pea grains is shown in Fig 5.6. The relationship between the porosity and moisture content observed is expressed in Eq. 5.6 with $R^2 = 0.998$. A similar increasing in porosity was reported by Baryeh and Mangope (2003) for QP-38 variety of pigeon pea, Chowdhury et al. (2001) for gram and Nimkar and Chattopadhyay (2001) for green gram.

$$P = -0.004M^2 + 0.317M + 32.73, \quad (R^2 = 0.998) \quad \ldots \quad (5.6)$$

Where,

- $P$ = Porosity, %
- $M$ = Moisture content, % (d.b.)

![Fig. 5.6 Effect of moisture content on porosity of pigeon pea grains](image-url)

5.1.7 Effect on angle of repose of pigeon pea grain
The experimental results for the angle of repose with respect to moisture content are given in Table 5.2. The values were found to increase from 28.17° to 34.08° in the moisture range of 10 to 30% (d.b.). The variation in angle of repose with moisture content of pigeon pea grains is shown in Fig 5.7. The relationship observed between the angle of repose and moisture content is given in Eq. 5.7 with $R^2 = 0.999$. The increasing trend of angle of repose could be due to the adhesion of grains having high moisture content which resulted into higher angle of repose. This is in accordance with the findings reported by Pradhan et al. (2008) in case of karanja kernels. A similar increasing in angle of repose with increase in moisture content was reported by Baryeh and Mangope (2003) for pigeon pea, Tavkoli et al. (2009) for soybean, Chowdhury et al. (2001) for gram and Nimkar and Chattopadhyay (2001) for green gram.

$$\theta = 0.005M^2 + 0.063M + 26.93, \quad (R^2 = 0.999)$$

*(\text{5.7})*

Where,

$\theta$ = Angle of repose, degree

$M$ = Moisture content, % (d.b.)

![Fig. 5.7 Effect of moisture content on angle of repose of pigeon pea grains](image-url)

5.1.8 Effect on static coefficient of friction of pigeon pea grain
The static coefficients of friction for pigeon pea, determined with respect to galvanized, plywood and glass surfaces are presented in Table 5.2. At all the moisture contents, the static coefficient of friction was highest against plywood surface which ranged from 0.41 to 0.62, for galvanized sheet from 0.34 to 0.52 and lowest for glass surface that is from 0.336 to 0.456. The grain may become more adhesive and sliding characteristics decreased with increasing the moisture content, so that the static coefficient of friction increased. The variation of static coefficient of friction against three surfaces with moisture content for pigeon pea grains is shown in Fig. 5.8. The relationship between the static coefficient of friction against galvanized sheet, plywood and glass surfaces and moisture content had $R^2$ value of 0.9977, 0.9930 and 0.9975, respectively as expressed in Eq. 5.8a, 5.8b and 5.8c. A similar increasing in angle of repose was reported by Baryeh and Mangope (2003) for pigeon pea.

For galvanized sheet, $\mu_{ga} = -0.0001M^2 + 0.0154M + 0.2660$ ($R^2 = 0.9977$) ... (5.8a)

For plywood, $\mu_{pl} = -0.0001M^2 + 0.0134M + 0.2200$ ($R^2 = 0.9930$) ... (5.8b)

For glass surface, $\mu_{gl} = 0.0002M^2 - 0.0023M + 0.3374$ ($R^2 = 0.9975$) ... (5.8c)

Where $\mu_{ga}$, $\mu_{pl}$ and $\mu_{gl}$ are the static coefficient of galvanized, plywood and glass surfaces, respectively.
Fig. 5.8 Effect of moisture content on static coefficient of friction with different surfaces

5.2 Proximate Composition of Pigeon pea Grain

The data obtained for the proximate composition of pigeon pea i.e., moisture content, protein, carbohydrate, fat, crude fibre and total ash are presented in Table 5.3. All the determinations were replicated thrice and average values are reported. The moisture content of pigeon pea grains was found to be 10.46 ± 0.51% (w.b.), protein 18.73 ± 0.24 %, carbohydrate 58.15 ± 0.09 %, fat 1.62 ± 0.12 %, crude fibre 7.45 ± 0.18 %, total ash 3.70 ± 0.10 %. These results of proximate composition of pigeon pea grain were observed in agreement with the results reported by Faris and Singh (1990).
Table 5.3 Proximate composition of pigeon pea grain

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Average value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content, (w.b.)</td>
<td>10.46 ± 0.51</td>
</tr>
<tr>
<td>Protein</td>
<td>18.73 ± 0.24</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>58.15 ± 0.09</td>
</tr>
<tr>
<td>Fat</td>
<td>01.62 ± 0.12</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>07.45 ± 0.18</td>
</tr>
<tr>
<td>Total Ash</td>
<td>03.70 ± 0.10</td>
</tr>
</tbody>
</table>

5.3 Results of Oil Treatment (Control)

The oil treatment allows oil to penetrate the pigeon pea grains through the husk into the cotyledon layer. Oil contains neutral lipids and free fatty acids. The negatively charged polar group of fats may interact with positively charged protein of upper layer of cotyledons and cause loosening of seed coat (Srivastava, 1997). The raw data on the husk removed, hulling efficiency, protein content and cooking time of the oil treated pigeon pea grains are given in Appendix D. The average value of husk removed, hulling efficiency, protein content and cooking time were found to be 76.30 %, 76.25 %, 19.12 % and 26.8 min, respectively.

5.4 Standardization of Enzyme Proportion

The average value regarding the husk removed and hulling efficiency obtained while standardizing the three enzymes at different proportions are given in Table 5.4. It is clear from the table that the maximum husk removed and hulling efficiency for xylanase, pectinase and cellulase in the proportion of 2: 1: 1 (xylanase 50 %: pectinase 25 %: cellulase 25 %) were 90.31 % and 86.29 %, respectively followed by 81.99 % hulling efficiency in case of above enzymes proportion 1: 1: 1. The minimum hulling efficiency was found as 79.43 % in the enzyme proportion 1: 1: 2. Therefore, enzyme proportion 2: 1: 1 was taken for the enzymatic treatment. The maximum hulling efficiency at 50 % xylanase enzyme proportion might be due to the husk was rich in non starchy
polysaccharides and contained varying amounts of arabinose and xylose in most of the fraction (Swami et al., 1991).

Table 5.4 Effect of different enzyme proportion on husk removed and hulling efficiency

<table>
<thead>
<tr>
<th>Enzyme proportion X : P : C</th>
<th>Husk (g)</th>
<th>Broken (g)</th>
<th>Powder (g)</th>
<th>Unhulled (g)</th>
<th>Dhal (g)</th>
<th>Husk removed (%)</th>
<th>Hulling efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 1 : 1</td>
<td>221.95</td>
<td>34.68</td>
<td>40.21</td>
<td>273.59</td>
<td>1426.3</td>
<td>82.77±0.56</td>
<td>81.99±0.8</td>
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<tr>
<td>2 : 1 : 1</td>
<td>241.83</td>
<td>64.03</td>
<td>62.64</td>
<td>127.83</td>
<td>1497.6</td>
<td>90.31±1.67</td>
<td>86.29±1.1</td>
</tr>
<tr>
<td>1 : 2 : 1</td>
<td>229.62</td>
<td>62.42</td>
<td>69.3</td>
<td>216.78</td>
<td>1417.2</td>
<td>85.69±1.10</td>
<td>81.56±0.5</td>
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<tr>
<td>1 : 1 : 2</td>
<td>226.23</td>
<td>77.78</td>
<td>74.11</td>
<td>235.46</td>
<td>1379.2</td>
<td>84.53±1.21</td>
<td>79.43±0.6</td>
</tr>
<tr>
<td>3 : 1 : 1</td>
<td>216.31</td>
<td>93.41</td>
<td>50.09</td>
<td>223.11</td>
<td>1412.0</td>
<td>80.74±1.36</td>
<td>80.62±1.4</td>
</tr>
</tbody>
</table>

X=Xylanase, P=Pectinase, C=Cellulase

5.5 Effect of Enzymes on Microstructure of Pigeon pea Grains

Sectional images of enzyme treated pigeon pea grain samples obtained using scanning electron microscope at 540 K magnification are shown in Plate 5.1(a-e). Efforts were made to estimate the thickness of cavity formed between husk and cotyledon after enzymatic hydrolysis of samples of pigeon pea grains. The cavities are indicated by arrows in the above plates. Thickness of cavity was measured at three different places on each image, using software incorporated in scanning electron microscope during image analysis. The average values of three different observations were considered to represent the estimated cavity thickness in each image. Enzyme treated sectional images (1 to 30) showed the gum layer between husk and endosperm hydrolysed due to enzymatic action and formation of clear gap between husk and endosperm. It was observed from the images that as the thickness of cavity increased, the percentage of husk removed also increased. The husk and endosperm on the images appeared white or gray due to reflection of electron beam while the cavities or empty spaces on the surface black.
Plate 5.1a Sectional images of pigeon pea grains of enzymatic treatments (1 to 6)
Plate 5.1b Sectional images of pigeon pea grains of enzymatic treatments (7 to 12)
Treatment 13  (Cavity thickness = 14.23 µm)

Treatment 14 (Cavity thickness = 11.93 µm)

Treatment 15 (Cavity thickness = 12.18 µm)

Treatment 16 (Cavity thickness = 11.49 µm)

Treatment 17 (Cavity thickness = 48.84 µm)

Treatment 18 (Cavity thickness = 8.49 µm)
Plate 5.1c Sectional images of pigeon pea grains of enzymatic treatments (13 to 18)

Treatment 19 (Cavity thickness = 17.77 µm)

Treatment 20 (Cavity thickness = 14.67 µm)

Treatment 21 (Cavity thickness = 8.54 µm)

Treatment 22 (Cavity thickness = 3.80 µm)
Plate 5.1d Sectional images of pigeon pea grains of enzymatic treatments (19 to 24)
Plate 5.1e Sectional images of pigeon pea grains of enzymatic treatments (24 to 30)

Images of unhydrolyzed grains (without treatment) and control (oil treatment) are shown in Plate 5.2. In the images of unhydrolysed grain sample, sharply adhered husk surrounded by endosperm were observed. No isolated cavities were visible. The husk was attached with endosperm by gum layer. Progressive biodegradation of gum layer was visible through their cavities in case of oil treated samples (Plate OT\textsubscript{1} and OT\textsubscript{2}). It could be seen from the images that some portion of husk remained intact with endosperm after oil treatment which reduced the percentage of husk removed.

5.5.1 Comparison of cavity thickness with husk removed

Efforts were made to quantify the microstructural changes due to enzymatic treatment by estimating a representative cavity thickness to correlate it with the percentage husk removed. The data on cavity thickness at different enzymatic treatment and percentage husk removed are given in Table 5.5. From the table it is clear that as the cavity thickness increased, the percentage husk removed also increased. The cavity thickness varied from 3.80 to 48.84 \( \mu \text{m} \). The minimum cavity thickness was found for treatment number 22, having the combination of enzyme concentration of 40 mg/100g dry matter, 9 h incubation time, 40 °C incubation temperature and 5.0 tempering water pH. The maximum cavity thickness was found for treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH. The cavity thickness was recorded comparatively lower in the treatment number 18 having minimum level of enzyme concentration. Also, the treatment number 21, 22, 23 and 24 gave lower values of cavity thickness. This could be due either the higher levels of incubation temperature or the tempering water pH. This could indicate the profound effect of selected levels of hydrolysis parameters.

The husk removed varied from 80.81 to 94.23 % (Table 5.5). The minimum husk removed was found for treatment number 24 having the
combination of enzyme concentration of 40 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 4.0 tempering water pH followed by treatment number 22. The maximum husk removed was obtained in treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH. This indicated that with the increase in cavity thickness increased the husk removed also for the enzyme treated samples.
WT = Without oil treatment      OT = With oil treatment

**Plate 5.2** Sectional images of pigeon pea grains with and without oil treatment

**Table 5.5** Effect of various levels of enzymatic and oil treatment on cavity thickness and husk removed

<table>
<thead>
<tr>
<th>Treat. No.</th>
<th>Enzyme concentration (mg/100 g dry matter)</th>
<th>Incubation Time (h)</th>
<th>Incubation Temperature (°C)</th>
<th>Temperature water pH</th>
<th>Cavity thickness (µm)</th>
<th>Husk removed (%)</th>
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<tbody>
<tr>
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<td>26.57</td>
<td>90.69</td>
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<td>9</td>
<td>50</td>
<td>5.0</td>
<td>31.55</td>
<td>92.02</td>
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<td>89.35</td>
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<td>9</td>
<td>50</td>
<td>5.0</td>
<td>21.67</td>
<td>90.24</td>
</tr>
</tbody>
</table>
For oil treatment (control), cavity thickness of 6.07 µm and 3.84 µm and corresponding husk removed as 77.22% and 74.65% were found in samples of oil treated samples OT\textsubscript{1} and OT\textsubscript{2}, respectively. The minimum cavity thickness and percentage husk removed were observed in case of oil treated samples as compared to enzymatic treatment. Moreover, in all the enzymatic treatments, except treatment 22 and 24, the cavity thickness values were higher than the oil treatment (control).

The relationship between cavity thickness and percentage husk removed for enzymatic treatment samples is shown in Fig. 5.9. It was observed from the Fig. 5.9 that the cavity thickness of enzymatic treated samples increased which resulted in to the increase in the percentage husk removed. This might be due to the complete hydrolysis of gum layer which increased the cavity thickness that resulted into increase in the separation of husk from the cotyledon and the percentage of husk removed.

![Fig. 5.9 Relationship between cavity thickness and percentage husk removed by enzymatic treatments](image-url)
5.6 Effect of Enzymatic Treatment on Hulling Efficiency

The analysis of variance (ANOVA) was made for the experimental data and the significance of enzyme concentration, incubation time, incubation temperature and tempering water pH as well as their interactions on hulling efficiency were analyzed. The response surface quadratic model was fitted to the experimental data and statistical significance of linear, interaction and quadratic effects were analyzed for hulling efficiency response (Table 5.6).

The results showed that among linear effects, incubation temperature and tempering water pH had significant effect on hulling efficiency \((p<0.05)\) at 5\% and \((p>0.01)\) 1 \% level of significance, respectively. However, linear effects of enzyme concentration, incubation time and interaction effects of enzyme concentration, incubation time, incubation temperature and tempering water pH were found to be non significant. Quadratic effect of enzyme concentration and incubation temperature had significant effect on hulling efficiency \((p<0.01)\) at 1\% level of significance while effect of tempering water pH on hulling efficiency \((p<0.05)\) at 5\% level of significance. The incubation time was found to be non significant on hulling efficiency.

The hulling efficiency varied from 76.95 to 88.95 % (Table 5.7). The raw data regarding weight of different fractions, actual husk removed and hulling efficiency obtained with different combination of variables are given in Appendix E. The minimum hulling efficiency was found in treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH, while the maximum hulling efficiency found in treatment number 8 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 45 °C incubation temperature and 5.5 tempering water pH. The maximum husk was removed in treatment number 17 due to higher level of enzyme concentration (60 mg/100g dry matter) leading to the excessive hydrolytic activities which affected the internal structure of the grain. This phenomenon led to higher breakage of grain which reduced the hulling efficiency. The quadratic response surface model data indicated the results as significant. The coefficient of determination \((R^2)\) was 0.9062 for enzymatic pre-treatment which indicated
that the model could fit the data for enzyme activity very well for all the four variables, i.e., enzyme concentration, incubation time, incubation temperature and tempering water pH.

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

Hulling efficiency, % = 87.44 -0.86X_1 - 0.43X_2 - 1.11X_3 + 1.71X_4 - 0.18X_1X_2 - 0.096X_1X_3 - 0.10X_1X_4 + 0.091X_2X_3 - 0.15X_2X_4 + 0.35X_3X_4 - 1.51X_1^2 - 0.50X_2^2 - 1.50X_3^2 - 1.07X_4^2

----(5.9)

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 5.6 ANOVA for effect of enzymatic treatment variables on hulling efficiency

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Sum of Square</th>
<th>F Value</th>
<th>p-value Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>247.94</td>
<td>17.71</td>
<td>2.58*</td>
<td>0.0395</td>
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<td>X_1: Enzyme concentration</td>
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<td>17.61</td>
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<td>X_2: Incubation time</td>
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<td>4.42</td>
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<td>X_3: Incubation temperature</td>
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<td>29.70</td>
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</tr>
<tr>
<td>X_4: Tempering water pH</td>
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<td>70.52</td>
<td>70.52</td>
<td>10.28**</td>
<td>&lt;0.0059</td>
</tr>
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<td>62.78</td>
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<td>61.54</td>
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* and ** indicate significant at 5 % and 1 % level of significance, respectively

Table 5.7 Effect of enzymatic treatment variables on hulling efficiency, protein content and cooking time

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<th>Response</th>
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<td>9</td>
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5.6.1 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 constant at 50 °C and 5.0, respectively which is shown in Fig. 5.10. It could be observed that with increase in incubation time, the hulling efficiency increased at a particular enzyme concentration. It also confirms the findings that hulling efficiency first increases with incubation time 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 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.

The minimum hulling efficiency of 76.95 % was obtained for the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH whereas, the maximum hulling efficiency was found for the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 45 °C incubation temperature and 5.5 tempering water pH. This showed that incubation temperature and tempering water pH played prominent role than the enzyme concentration and incubation time on hulling efficiency.

5.6.2 Effect of enzyme concentration and temperature on hulling efficiency

The effect of enzyme concentration and incubation temperature on hulling efficiency was determined keeping incubation time and tempering water pH constant at 9 h and 50 °C, respectively which is shown in Fig. 5.11. Three dimensional responses for hulling efficiency of enzyme treated samples were generated. From these surfaces, it could be evident that hulling efficiency 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

| Design points above predicted value | 88.95 |
| Design points below predicted value | 76.95 |

X_1 = Enzyme concentration  
X_2 = Incubation time  

Actual Factors  
X_3 = Incubation temperature  
X_4 = Tempering water pH

**Fig. 5.10 Effect of enzyme concentration and incubation time on hulling efficiency**
**Fig. 5.11 Effect of enzyme concentration and incubation temperature on hulling efficiency**

Temperature had shown significant effect on hulling efficiency. It was observed that with increase in incubation temperature, the hulling efficiency increased at a particular enzyme concentration. The reduction in enzymatic activity at above optimum temperature was due to denaturing of enzyme, resulting in the reduction of the hulling efficiency. It also confirmed the facts that maximum enzymatic reaction occurred at optimum temperature levels.

**5.6.3 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 time and incubation temperature constant at 9 h and 5.0, respectively as shown in Fig. 5.12. Three dimensional responses for hulling efficiency of enzyme treated samples were generated. From these surfaces, it could be evident that hulling efficiency initially increased with increase in tempering water pH and enzyme concentration and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis.
parameters within the selected range. Tempering water pH had significant effect on hulling efficiency and a sharp increase in hulling efficiency up to 5.49 pH value. It could be observed that with increase in tempering water pH, the hulling efficiency increased at a particular enzyme concentration. The reduction in enzymatic activity at above optimum pH was due to denaturing of enzymes, resulting in a decrease in the hulling efficiency.

5.6.4 Effect of incubation time and incubation temperature on hulling efficiency

The effect of incubation time and incubation temperature on hulling efficiency at constant enzyme concentration (40 mg/100g) and tempering water pH (5.0) is shown in Fig. 5.13. Three dimensional responses for hulling efficiency of enzyme treated samples were generated. From these surfaces, it could be evident that hulling efficiency initially increased with increase in incubation time and incubation temperature and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters.

![Fig. 5.12 Effect of enzyme concentration and tempering water pH on hulling efficiency](image-url)
Fig. 5.13 Effect of incubation time and incubation temperature on hulling efficiency

within the selected range. Incubation temperature was showing significant effect on hulling efficiency. However, it was observed that the hulling efficiency increased with increase in incubation time was small as compared to incubation temperature. It was also observed that with increase in incubation temperature, the hulling efficiency increased at a particular incubation time. The reduction in enzyme activity at above optimum incubation temperature would denature the enzymes, resulting in a decrease in the hulling efficiency

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

The effect of incubation time and tempering water pH on hulling efficiency at constant enzyme concentration (40 mg/100g) and incubation temperature (50 °C) is shown in Fig. 5.14. Three dimensional responses for hulling efficiency of enzyme treated samples were generated. It could be observed that with increase in tempering water pH, the hulling efficiency increased at a particular incubation time. From these surfaces, it is evident that hulling efficiency initially increased
with increase in incubation time and tempering water pH and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Effect of pH on hulling efficiency was found significant. However, incubation time and interaction of these two factors were found to be non-significant. It was also observed from the Fig. 5.14 that the increase in hulling efficiency with the increase in incubation time within the range tested was small.

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

The effect of incubation temperature and tempering water pH on hulling efficiency at constant enzyme concentration (40 mg/100g) and incubation time (9 h) is shown in Fig. 5.15. Three dimensional responses for hulling efficiency of enzyme treated samples were generated. It was observed that with increase in tempering water pH, the hulling efficiency increased at a particular incubation temperature. From these surfaces, it could be evident that hulling efficiency initially increased with increase in incubation temperature and tempering water pH and then started decreasing, thereby indicating the

Fig. 5.14 Effect of incubation time and tempering water pH on hulling efficiency
Fig. 5.15 Effect of incubation temperature and tempering water pH on hulling efficiency

existence of optimum levels of hydrolysis parameters within the selected range. Effect of tempering water pH and incubation temperature on hulling efficiency was found significant. However, interaction of these two factors were found to be non-significant.

5.7 Effect of Enzymatic Treatment on Protein Content

The response surface quadratic model implied the significant effect of selected enzymatic treatments on protein content of pigeon pea dhal. The analysis of variance (ANOVA) was carried out for the experimental data and the significance of enzyme concentration, incubation time, incubation temperature and tempering water pH as well as their interactions on protein content (Table 5.8). The results showed that among linear effects, enzyme concentration and incubation time had significant effect on protein content (p<0.01) at 1% level of significance. However, linear effects of incubation temperature, tempering water pH and interaction effects of enzyme concentration, incubation time, incubation
temperature and tempering water pH were found to be non-significant. Quadratic effect of enzyme concentration had significant effect on protein content (p<0.01) at 1% level of significance while effects of incubation time, incubation temperature and tempering water pH were found to be non-significant on protein content.

The protein content of enzyme treated pigeon pea dhal ranged from 19.30 to 21.81% (Table 5.7). The minimum protein content was found for treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH whereas, the maximum protein content was found in treatment number 4 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 55 °C incubation temperature and 5.5 tempering water pH. The response surface quadratic model data indicated the results as significant. The coefficient of determination (R²) and CV % value for protein content were 0.9062 and 1.61, respectively.

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<th>p-value</th>
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<td>5.90**</td>
<td>0.0008</td>
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<td>X₁: Enzyme concentration</td>
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<td>&lt;0.0001</td>
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<td>X₂: Incubation time</td>
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</tr>
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<td>X₃: Incubation temperature</td>
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<td>X₄: Tempering water pH</td>
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<td>1.806E-003</td>
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<td>0.9014</td>
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<td>1.406E-003</td>
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</tr>
<tr>
<td>X₂X₄</td>
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<td>0.019</td>
<td>0.019</td>
<td>0.17</td>
<td>0.6892</td>
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</table>
The response surface equation was obtained for the model of second degree in terms of coded factors is as under.

Protein content, % = 21.40 - 0.36X_1 - 0.34X_2 - 0.097X_3 + 0.12X_4 + 0.011X_1X_2 + 0.052X_1X_3 + 0.023X_1X_4 + 9.375\times10^{-3}X_2X_3 - 0.034X_2X_4 + 0.14X_3X_4 - 0.29X_1^2 - 0.14X_2^2 - 0.077X_3^2 - 0.029X_4^2 \quad \text{---(5.10)}

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

5.7.1 Effect of enzyme concentration and incubation time on protein content

The effect of enzyme concentration and incubation time on protein content was determined keeping incubation temperature and tempering water pH constant at 50 °C and 5.0, respectively which is shown in Fig. 5.16. It could be observed that with increase in incubation time, the protein content increased at a particular enzyme concentration. Initial increase may be due to addition of enzymes (proteins). Gradual decrease may be due to hydrolytic action of constitutive proteolytic enzyme already present in outermost layer of endosperm. Higher incubation time may have produced protease inhibitor substances for enzyme action resulting in lower protein content (Singh and Eggum, 1984)

The minimum protein content of 19.30 % was obtained in the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C
incubation temperature and 5.0 tempering water pH whereas, the maximum protein content of 21.81 % was found at the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 55 °C incubation temperature and 5.5 tempering water pH. These shows that enzyme concentration and incubation time are playing prominent role than the hydrolysis temperature and tempering water pH on protein content.

5.7.2 Effect of enzyme concentration and Incubation temperature on protein content

The effect of enzyme concentration and incubation temperature on protein content was determined keeping incubation time and tempering water pH constant at 9 h and 50 °C, respectively which is shown in Fig. 5.17. Three dimensional responses for protein content of enzyme treated samples were generated. From these surfaces, it could be evident that protein content initially increased with increase in tempering water pH and enzyme concentration and
Fig. 5.17 Effect of enzyme concentration and incubation temperature on protein content

then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Enzyme concentration had shown significant effect on protein content. It was observed that with increase in temperature, the protein content increased at a particular enzyme concentration.

5.7.3 Effect of enzyme concentration and tempering water pH on protein content

The effect of enzyme concentration and tempering water pH on protein content was found keeping incubation time and incubation temperature constant at 9 h and 50 °C, respectively which is shown in Fig. 5.18. Three dimensional responses for protein content of enzyme treated samples were generated. From these surfaces, it could be evident that protein content initially increased with increase in tempering water pH and enzyme concentration and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. The enzyme concentration had highly significant on protein content (p< 0.01) at 1 % level of significance whereas the
tempering water pH and the interaction effect of enzyme concentration and tempering water pH on protein content was found to be non-significant.

5.7.4 Effect of incubation time and incubation temperature on protein content

The effect of incubation time and incubation temperature on protein content at constant enzyme concentration (40 mg/100g) and tempering water pH (5.0) is shown in Fig. 5.19. Three dimensional responses for protein content of enzyme treated samples were generated. From these surfaces, it could be evident that protein content initially increased with increase in incubation time and incubation temperature and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. Incubation temperature had shown non-significant effect on protein content. However, it was observed that the protein content increased with increase in incubation time and incubation temperature was small.

![Fig. 5.18 Effect of enzyme concentration and tempering water pH on protein content](image-url)
5.7.5 Effect of incubation time and tempering water pH on protein content

The effect of incubation time and tempering water pH on protein content at constant enzyme concentration (40 mg/100g) and incubation temperature (50 °C) is shown in Fig. 5.20. Three dimensional responses for protein content of enzyme treated samples were generated. It could be observed that with increase in tempering water pH, the protein content increased linearly at a particular incubation time. From these surfaces, it could be evident that protein content initially increased with increase in tempering water pH and decreased with increase in incubation time. Effect of incubation time on protein content was found highly significant (p<0.01) at 1 % level of significance whereas the tempering water pH and the interaction effect of incubation time and tempering water pH were found to be non-significant.
5.7.6 Effect of incubation temperature and tempering water pH on protein content

The effect of incubation temperature and tempering water pH on protein content at constant enzyme concentration (40 mg/100g) and incubation time (9 h) is shown in Fig. 5.21. Three dimensional responses for protein content of enzyme treated samples were generated. It could be observed that with increase in tempering water pH, the protein content increased at a particular incubation temperature. From these surfaces, it could be evident that protein content increased with increase in incubation temperature and tempering water pH and then started decreasing, thereby indicating the existence of optimum levels of hydrolysis parameters within the selected range. The linear and interaction effect of tempering water pH and incubation temperature were found to be non-significant.

Fig. 5.20 Effect of incubation time and tempering water pH on protein content
5.8 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 tempering water pH as well as their interactions on cooking time of enzyme treated pigeon pea dhal were analyzed (Table 5.9). The results showed that among linear effects, enzyme concentration and tempering water pH had 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 5.9). However, linear effects of incubation temperature and interaction effects of enzyme concentration, incubation time, incubation temperature and tempering water pH 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 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 5.7). The minimum cooking time was found in treatment number 5 having the combination of enzyme concentration of 50 mg/100g dry matter, 12 h incubation time, 45 °C incubation temperature and 5.5 tempering water pH whereas, the maximum cooking time was found in experiment number 20 having the combination of enzyme concentration of 40 mg/100g dry matter, 3 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH. The coefficient of determination (R²) and CV % values for cooking time were 0.9062 and 1.88, respectively.

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

\[
\text{cooking time, min} = 22.00 - 0.25X_1 - 0.33X_2 + 0.083X_3 - 0.25X_4 - 0.19X_1X_2 \\
- 0.062X_1X_3 + 0.000X_1X_4 - 0.12X_2X_3 - 0.19X_2X_4 + 0.19X_3X_4 + 0.23X_1^2 + \\
0.29X_2^2 + 0.29X_3^2 + 0.10X_4^2
---(5.11)\
\]

Where, \(X_1 =\) Enzyme concentration (mg/100g dry matter), \(X_2 =\) Incubation time (h), \(X_3 =\) Incubation temperature (°C) and \(X_4 =\) Tempering water pH
<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Sum of Square</th>
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<th>p-value Prob&gt;F</th>
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<td>$X_4$: Tempering water pH</td>
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<td>2.33</td>
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</table>

* and ** indicate significant at 5% and 1% level of significance, respectively
5.8.1 Effect of enzyme concentration and incubation time on cooking time

The effect of enzyme concentration and incubation time on cooking time was determined keeping incubation temperature and tempering water pH constant at 50 °C and 5.0, respectively which is shown in Fig. 5.22. 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 boimolecules (polymer) into simple precursors. It also affected the relative proportion of other biomolecules which might lead to decrease the cooking time. Prolonged exposr of grain to enzymes might have increased the cooking time because of hardening effect due to combined effect of temperature and moisture. The individual effect of enzyme concentration and incubation time on cooking time was found significant at 1 % and 5 % level of significance, respectively. However, their interaction effect was found non-significant.

The minimum cooking time of 21.5 min was obtained at the combination of enzyme concentration of 50 mg/100g 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/100g dry matter, 3 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH. These shows that incubation time is playing prominent role for variation in cooking time.

5.8.2 Effect of enzyme concentration and Incubation temperature on cooking time

The effect of enzyme concentration and incubation temperature on cooking time was determined keeping incubation time and tempering water pH constant at 9 h and 5.0, respectively which is shown in Fig. 5.23. Three
Fig. 5.22 Effect of enzyme concentration and incubation time on cooking time

Fig. 5.23 Effect of enzyme concentration and incubation temperature on cooking time
dimensional responses for cooking time of enzyme treated samples were generated. From these surfaces, 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 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 influenced the cooking time of legumes as reported by Muller 1967. Also, the findings are in accordance with the results obtained by Singh and Rao (1995) who reported that the pectinase treatment decreased the cooking time as compared to other enzymes.

5.8.3 Effect of enzyme concentration and tempering water pH on cooking time

The effect of enzyme concentration and tempering water pH on cooking time was determined keeping incubation time and incubation temperature constant at 9 h and 50 °C, respectively which is shown in Fig. 5.24. Three dimensional responses for cooking time of enzyme treated 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 and enzyme concentration had shown significant effect on cooking time (p<0.05) at 5 % level of significance while interaction effect of enzyme concentration and tempering water pH on cooking time was found to be non-significant.
Fig. 5.24 Effect of enzyme concentration and tempering water pH on cooking time

Fig. 5.25 Effect of incubation time and incubation temperature on cooking time
5.8.4 Effect of incubation time and incubation temperature on cooking time

The effect of incubation time and incubation temperature on cooking time at constant enzyme concentration (40 mg/100g) and tempering water pH (5.0) is shown in Fig. 5.25. Three dimensional responses for cooking time of enzyme treated samples were generated. From these surfaces, it could be evident 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 non-significant effect on cooking time. Prolonged exposure of grain to enzymes might be increased cooking time because of hardening effect of pigeon pea grain due to combined effect of temperature and moisture.

5.8.5 Effect of incubation time and tempering water pH on cooking time

The effect of incubation time and tempering water pH on cooking time at constant enzyme concentration (40 mg/100g) and incubation temperature (50 °C) is shown in Fig. 5.26. Three dimensional responses for cooking time of enzyme treated samples were generated. It could be observed that with increase in tempering water pH, 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 and incubation time and then started increasing. Effect of incubation time and tempering water pH on cooking time was found significant at 1% and 5% level of significance, respectively. However, interaction of these two factors was found to be non-significant.

5.8.6 Effect of incubation temperature and tempering water pH on cooking time

The effect of incubation temperature and tempering water pH on cooking time at constant enzyme concentration (40 mg/100g) and incubation time (9 h) is shown in Fig. 5.27. Three dimensional responses for cooking time of enzyme treated samples were generated. It could be observed that with
Fig. 5.26 Effect of incubation time and tempering water pH on cooking time
Fig. 5.27 Effect of incubation temperature and tempering water pH on cooking time

increase in tempering water pH, the cooking time decreased at a particular incubation temperature. Tempering water pH had shown 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.

5.9 Optimization of Enzymatic Treatment Variables

In case of response surface analysis, the selected response surface quadratic model was explained in section 4.11.2. 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 values of enzymatic hydrolysis pre-treatment were evaluated using equations 5.9, 5.10 and 5.11. The multiple regression package was used for this purpose. 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 (48.5 °C) and tempering water pH 5.49 which gave the predicted values of hulling efficiency 88.37 %, protein content 21.57 % and cooking time 21.91 min. The optimum values for different variables and their predicted responses thus obtained are given in Table 5.10.
Table 5.10 Optimized variables and their responses for enzymatic pre-treatment of pigeon pea grains

<table>
<thead>
<tr>
<th>Variables</th>
<th>Optimized values</th>
<th>Responses</th>
<th>Predicted values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme concentration mg/100 g dry matter</td>
<td>37.80</td>
<td>Hulling efficiency, %</td>
<td>88.37</td>
</tr>
<tr>
<td>Incubation time, h</td>
<td>8.69</td>
<td>Protein Content, %</td>
<td>21.57</td>
</tr>
<tr>
<td>Incubation temperature, °C</td>
<td>48.48</td>
<td>Cooking time, min</td>
<td>21.91</td>
</tr>
<tr>
<td>Tempering water pH</td>
<td>5.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.9062</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

5.10 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. The raw data regarding weight of different fractions, actual husk removed and hulling efficiency obtained at optimized condition of variables are given in Appendix F. The average experimental values of hulling efficiency, protein content and cooking time are presented in Table 5.11. From the above table, it could be seen that the predicted values of hulling efficiency, protein content and cooking time were 88.37 %, 21.57 % and 21.91 min, respectively. These were experimentally verified in the laboratory and observed values of hulling efficiency, protein content and cooking time were found to be 88.12 %, 21.81 % and 21.50 min, respectively. The predicted values of hulling efficiency, protein content and cooking time obtained from equations showed 0.28, 1.10 and 2.19 % deviation from the experimental values, respectively. It could reveal that the experimental values were very close to the predicted values which confirmed the optimum conditions.
Table 5.11 Predicted and experimental values of responses at optimum levels of different variables

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Responses</th>
<th>Predicted values</th>
<th>Experimental values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hulling efficiency, %</td>
<td>88.37(0.28)</td>
<td>88.12</td>
</tr>
<tr>
<td>2</td>
<td>Protein content, %</td>
<td>21.57(1.10)</td>
<td>21.81</td>
</tr>
<tr>
<td>3</td>
<td>Cooking time, min</td>
<td>21.97(2.19)</td>
<td>21.50</td>
</tr>
</tbody>
</table>

Figures in ( ) are per cent deviation from experimental values.

5.11 Comparison of Enzymatic and Oil Pre-treatment

The hulling efficiency, protein content and cooking time of oil treated (control) sample was found 76.25 %, 19.12 % and 26.8 min, respectively while the observed values of hulling efficiency, protein content and cooking time at the optimum conditions of enzymatic pre-treatment variables was 88.12 %, 21.81 % and 21.50 min, respectively. Hence, there was an increase in hulling efficiency of 13.47 %, Protein content of 12.33 % and 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 (1998) who reported that the enzyme treated dhal took 3 min less time in cooking over control.

5.12 Sensory Attributes of the Optimized Enzymatic Treatment and Oil Treatment (control) Cooked Blended Dhal

Sensory evaluation results of cooked blended dhal keeping same cooking time obtained from enzymatic and oil treatment are given in Table 5.12.
Cooked dhal and blended samples obtained from enzymatic and oil treatments, respectively is shown in Plate 5.3 and 5.4. 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. 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.

### Table 5.12 Effect 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>
Plate 5.3 Dhal obtained from enzymatic and oil pre-treatment samples

Plate 5.4 Cooked dhal and blended samples obtained from oil and enzymatic pre-treatment
These results are in agreement with the results reported by Singh and Rao (1995) regarding the acceptability score of dhal which was the highest for pectinase treated dhal followed by the control, solution of bicarbonate and mixture.

5.13 Recommended Enzymatic Pre-Treatment for Pigeon pea Milling

Based on the results reported in above sections, it could be recommended to follow enzymatic pre-treatment in place of prevailing oil milling pre-treatment. For enzymatic pre-treatment, the enzyme solution having 2:1:1 proportion of xylanase, pectinase and cellulase enzymes should be prepared using tempering water with pH of 5.49. The enzyme solution with pH of 5.49 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 values of hulling efficiency, protein content and cooking time at the suggested conditions of enzymatic pre-treatment variables were 88.12%, 21.81% and 21.50 min, respectively.

The enzymatic pre-treatment gave about 13.47% higher hulling efficiency as compared to traditionally practiced oil milling pre-treatment. The cooking time was reduced by 19.77% while the protein content of dehulled pigeon pea dhal was increased by 12.33% by following the suggested enzymatic pre-treatment of pigeon pea milling. The suggested flow chart for enzymatic pre-treatment method of pigeon pea milling for getting better recovery and quality of pigeon pea dhal is shown in Fig. 5.28. The quantity of enzymes required have been estimated considering the 10.46% (w.b.) moisture content of the pigeon pea normally used by the pulse mills.
Distilled water (21.0 ml) 
↓ 
pH adjustment to 5.49 
↓ 
Addition of enzymes 
(33.84 mg /100 g pigeon pea grain) 
(16.92 mg xylanase + 8.46 mg pectinase + 8.46 mg cellulase) 
↓ 
Enzyme solution (21.0 ml) 
↓ 
Addition of enzyme solution to 100 g cleaned and graded pigeon pea grain of 
(10.46 % (w.b.)moisture content ) 
↓ 
Moisture equilibrium 26.0 % (w.b.) m.c. 
↓ 
Enzyme concentration 
(37.80 mg /100 g dry matter) 
↓ 
Enzyme treated sample 
↓ 
Incubation for hydrolysis 
(Incubation temperature 48.5 \(^o\)C, Incubation time 8.69 h) 
↓ 
Drying (Tray dryer) 
(Drying at 60 \(^o\)C up to 10 ± 0.5 % (d.b.) m.c.) 
↓ 
Dehusking and splitting 
(using dhal mill) 
↓ 
Separation → Broken, husk, unhulled grains, powder 
↓ 
Dhal

Fig. 5.28 Suggested flow chart for enzymatic pre-treatment - method of pigeon pea milling
CHAPTER VI
SUMMARY AND CONCLUSIONS

Pigeon pea (*Cajanus cajan* L.) is one of the important pulse crops of India contributing 20.87% to the total production of all pulses. India accounts for 90% of the total world production of pigeon pea. The total area and production of pigeon pea in Gujarat state during 2010-11 were 0.277 million hectares and 0.273 million tonnes, respectively. The average yield was 986 kg/ha. In India, the annual production of pigeon pea in 2011-12 was 2.90 million tonnes from a cultivated area of 3.86 million hectares with the average yield of 751 kg/ha.

Pigeon pea is significantly contributing to meet the dietary requirement of crude fibre, ash, fat, magnesium, manganese and copper. Pigeon pea contains high amount of vitamin B, Carotene and ascorbic acid. These are deficient in cereals; therefore, pigeon pea has a good supplemental value of cereal based diet. Pigeon pea is consumed as dehulled splits, whole, canned, boiled, roasted or ground into flour to make a variety of desserts, snacks and main dishes. The cotyledons of dry grains excluding seed coat are called dhal. Pigeon pea is mainly consumed as dhal because it takes less time to cook and has acceptable appearance, texture, palatability, digestibility, and overall nutritional quality.

The pigeon pea grain is considered as most difficult for dehulling as compared to other pulses owing to its seed coat which is more firmly attached with the cotyledons through a layer of gum and mucilage. Due to the presence of gummy layer and hard seed coat, it is difficult to dehull. The primary objective of dehulling is to remove seed coat from the cotyledons, during which four different fractions, i.e., dhal, broken, powder and husk are obtained. During dehulling, noticeable amount of cotyledon material and germ are removed resulting into considerable loss.

Pre milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion. There are many milling methods
like wet milling, dry milling, CFTRI method, Pantnagar process, CIAE method and IIPR method developed for pigeon pea milling. There are various pre milling treatments, with respect to different milling methods, carried out before dehulling for loosening of seed coat of pigeon pea grains.

All the above mentioned treatments are time consuming, require almost 4 to 7 days for the complete milling of pigeon pea. Also, the survey work of few pulse mills in Gujarat revealed that the dry milling treatments carried out during the pulse milling for pigeon pea take longer processing time, about 7 to 8 days depending upon weather as sun drying is required to get satisfactory milling after pre-treatment. At present, the consumers’ requirements are that the dhal should be cooked well in minimum possible time and have a good taste and flavour. This necessitated the suitable pre-treatment for pigeon pea milling that can shorten the processing time and improve the product quality.

It is necessary to have special pre-treatment to dissolve the glue that binds the cotyledons of pigeon pea grains to the seed coat. It is evident that dehulling quality is highly dependent on physical properties of grains and pre-treatments. Enzymatic pre-treatments have shown increased recovery and quality of pigeon pea dhal.

In view of above, it was felt necessary to develop a suitable enzymatic pre-treatment which could be helpful in easy dehusking giving higher recovery of better nutritional, milling and cooking quality of dhal. Hence, the present research work was undertaken with the following objectives.

To study the physicochemical properties of pigeon pea grains.

To study the effect of enzymatic pre-treatments on loosening of seed coat of pigeon pea grains.

To study the milling quality of enzyme treated pigeon pea.

To determine the effect of enzymatic pre-treatments on protein content of dhal obtained through different effective treatments.
To study the cooking quality of dhal obtained through different effective treatments.

The experiments mainly consisted of physicochemical properties of pigeon pea grains, scanning electron microscopy of enzyme treated grains, enzymatic pre-treatments on milling quality, protein content and cooking time. All the experiments were carried out in the Department of Processing and Food Engineering, College of Agricultural Engineering & Technology, Junagadh Agricultural University, Junagadh and Department of Bio-chemistry, College of Agriculture, Junagadh Agricultural University, Junagadh.

The pigeon pea grain (Cv. BDN 2) used for the study was procured from Sagdividi farm of the Junagadh Agricultural University, Junagadh. Different properties of pigeon pea grains, namely size in terms of length, breath and thickness, sphericity, bulk density, porosity, true density, angle of repose and coefficient of static friction against different surfaces were determined as per the standard procedures. The proximate compositions of pigeon pea grains, viz., carbohydrate, protein, fat, crude fibre and ash content were determined at 10.46 %, (w.b.) moisture content.

The laboratory scale dehusking machine (for dhal milling) with capacity 85 kg/h was used for all the milling studies. Enzymatic pre treated and control samples of 2 kg weight having about 10 ± 0.5 % moisture content (w.b.) were milled using laboratory dehusking machine/dhal mill. Samples were milled at the standard settings of the machine, i.e., 1420 rpm operating speed and 64 kg/h feed rate. The different fractions of the milled product were separated for calculation of hulling efficiency. Generally, the dry 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 considered as control.

Selection of enzymes was based on the chemical composition and binding substances present between husk and cotyledon of pigeon pea grain. The xylanase, cellulase and pectinase are the key enzymes that rupture the binding materials leading to increase the dehulling efficiency. Preliminary trials were
under taken to arrive at standard ratio of enzymes, i.e., xylanase: pectinase : cellulase.

The effects of four enzymatic hydrolysis parameters viz., enzyme concentration (20, 30, 40, 50 and 60 mg/100 g dry matter), incubation time (3, 6, 9, 12 and 15 h), incubation temperature (40, 45, 50, 55 and 60 °C) and tempering water pH (4.0, 4.5, 5.0, 5.5 and 6.0) on hulling efficiency, protein content and cooking time were optimized using response surface methodology.

Microstructure of all the enzymatically hydrolyzed as well as oil treated (control) samples were examined using a Scanning Electron Microscope. Sensory evaluation of the cooked samples of enzyme treated and control samples immediately after cooking in terms of colour, appearance, flavour, texture, taste and overall acceptability was carried out.

The optimum conditions obtained through response surface analysis was verified by conducting the experiment in triplicates. The average value of dehulling efficiency, protein content and cooking time were considered for the validation.

Based on the inferences drawn from the results obtained during the study, the following conclusions could be drawn.

The average length, width, thickness, size and thousand grain mass of pigeon pea grains increased from 6.05 to 6.32 mm, 5.43 to 5.63 mm, 4.64 to 4.71 mm, 5.337 to 5.510 mm and 97.90 to 116.83 g with the increase in moisture content from 10 to 30 % (d.b.).

The sphericity was found to decrease from 0.883 to 0.871 with the increase in moisture content from 10 to 30 % (d.b.).

The bulk density and true density of pigeon pea grains decreased from 872 to 814 kg/m³ and 1353 to 1307 kg/m³ with increasing moisture content from 10 to 30 % (d.b.), respectively.

The porosity and angle of repose of pigeon pea grains increased from 35.47 to 37.96 % and 28.17° to 34.08° with increasing moisture content from 10 to 30 % (d.b.), respectively.
At all the moisture contents, the static coefficient of friction was highest against plywood surface which ranged from 0.41 to 0.62, for galvanized sheet from 0.34 to 0.52 and lowest for glass surface that is from 0.336 to 0.456.

The moisture content of pigeon pea grains was found to be 10.46 % (w.b.), protein 18.73 %, carbohydrate 58.15 %, fat 1.62 %, crude fibre 7.45 %, total ash 3.70 %.

The average value of husk removed, hulling efficiency, protein content and cooking time of oil treated pigeon pea grains were found to be 76.30 %, 76.25 %, and 19.12 % and 26.8 min, respectively.

The maximum husk removed and hulling efficiency for xylanase, pectinase and cellulase in the proportion of 2: 1: 1 (xylanase 50 %: pectinase 25 %: cellulase 25 %) were 90.31 % and 86.29 %, respectively followed by 81.99 % hulling efficiency in case of above enzymes proportion 1: 1: 1.

The cavity thickness observed through sectional images of enzyme treated pigeon pea samples using scanning electron microscope varied from 3.80 to 48.84 µm.

The minimum cavity thickness was found for treatment number 22, having the combination of enzyme concentration of 40 mg/100g dry matter, 9 h incubation time, 40 \(^\circ\)C incubation temperature and 5.0 tempering water pH. The maximum cavity thickness was found for treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 \(^\circ\)C incubation temperature and 5.0 tempering water pH.

The husk removed varied from 80.81 to 94.23 %. The minimum husk removed was found for treatment number 24 having the combination of enzyme concentration of 40 mg/100g dry matter, 9 h incubation time, 50 \(^\circ\)C incubation temperature and 4.0 tempering water pH followed by treatment number 22. The maximum husk removed was in case of treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 \(^\circ\)C incubation temperature and 5.0 tempering water pH. This showed
that the increase in cavity thickness increased the husk removed also for the enzyme treated samples.

For oil treatment (control), cavity thickness of 6.07 µm and 3.84 µm and husk removed as 77.22 % and 74.65 % were found in samples of oil treated samples OT₁ and OT₂, respectively. The cavity thickness and percentage husk removed were observed minimum in case of oil treated samples as compared to enzymatic treatment.

The hulling efficiency varied from 76.95 to 88.95 % obtained at different combination of variables. The minimum hulling efficiency was found in treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH, while the maximum hulling efficiency found in treatment number 8 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 45 °C incubation temperature and 5.5 tempering water pH.

The response surface equation for hulling efficiency was obtained as given below

\[
\text{Hulling efficiency,} \% = 87.44 - 0.86X₁ - 0.43X₂ - 1.11X₃ + 1.71X₄ - 0.18X₁X₂ - 0.096X₁X₃ - 0.10X₁X₄ + 0.091X₂X₃ - 0.15X₂X₄ + 0.35X₃X₄ - 1.51X₁² - 0.50X₂² - 1.50X₃² - 1.07X₄²
\]

Where,

\(X₁ = \text{Enzyme concentration (mg/100g dry matter), } X₂ = \text{Incubation time (h), } X₃ = \text{Incubation temperature (°C) and } X₄ = \text{Tempering water pH}\)

The protein content of enzyme treated samples ranged from 19.30 to 21.81 %. The minimum protein content was found for treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH whereas, the maximum protein content was found in treatment number 4 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 55 °C incubation temperature and 5.5 tempering water pH.

The response surface equation for protein content was obtained as given below

That the increase in cavity thickness increased the husk removed also for the enzyme treated samples.

For oil treatment (control), cavity thickness of 6.07 µm and 3.84 µm and husk removed as 77.22 % and 74.65 % were found in samples of oil treated samples OT₁ and OT₂, respectively. The cavity thickness and percentage husk removed were observed minimum in case of oil treated samples as compared to enzymatic treatment.

The hulling efficiency varied from 76.95 to 88.95 % obtained at different combination of variables. The minimum hulling efficiency was found in treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH, while the maximum hulling efficiency found in treatment number 8 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 45 °C incubation temperature and 5.5 tempering water pH.

The response surface equation for hulling efficiency was obtained as given below

\[
\text{Hulling efficiency,} \% = 87.44 - 0.86X₁ - 0.43X₂ - 1.11X₃ + 1.71X₄ - 0.18X₁X₂ - 0.096X₁X₃ - 0.10X₁X₄ + 0.091X₂X₃ - 0.15X₂X₄ + 0.35X₃X₄ - 1.51X₁² - 0.50X₂² - 1.50X₃² - 1.07X₄²
\]

Where,

\(X₁ = \text{Enzyme concentration (mg/100g dry matter), } X₂ = \text{Incubation time (h), } X₃ = \text{Incubation temperature (°C) and } X₄ = \text{Tempering water pH}\)

The protein content of enzyme treated samples ranged from 19.30 to 21.81 %. The minimum protein content was found for treatment number 17 having the combination of enzyme concentration of 60 mg/100g dry matter, 9 h incubation time, 50 °C incubation temperature and 5.0 tempering water pH whereas, the maximum protein content was found in treatment number 4 having the combination of enzyme concentration of 30 mg/100g dry matter, 6 h incubation time, 55 °C incubation temperature and 5.5 tempering water pH.

The response surface equation for protein content was obtained as given below
Protein content, % = 21.40 – 0.36X₁ - 0.34X₂ - 0.097X₃ + 0.12X₄
+0.011X₁X₂ + 0.052X₁X₃ + 0.023X₁X₄ +9.375E-003X₂X₃ - 0.034X₂X₄ +
0.14X₃X₄ - 0.29X₁² - 0.14X₂² - 0.077X₃² - 0.029X₄²

Where,
X₁= Enzyme concentration (mg/100g dry matter), X₂ = Incubation time (h), X₃ = Incubation temperature (⁰C) and X₄ = Tempering water pH

The cooking time varied from 21.5 to 24.5 min for different samples. The minimum cooking time was found in treatment number 5 having the combination of enzyme concentration of 50 mg/100g dry matter, 12 h incubation time, 45 ⁰C incubation temperature and 5.5 tempering water pH whereas, the maximum cooking time was found in treatment number 20 having the combination of enzyme concentration of 40 mg/100g dry matter, 3 h incubation time, 50 ⁰C incubation temperature and 5.0 tempering water pH.

The response surface equation for cooking time was obtained as given below

cooking time, min = 22.00 - 0.25X₁ - 0.33X₂ + 0.083X₃ - 0.25X₄ -
0.19X₁X₂ - 0.062X₁X₃ + 0.000X₁X₄ - 0.12X₂X₃ - 0.19X₂X₄ + 0.19X₃X₄ +
0.23X₁² + 0.29X₂² + 0.29X₃² + 0.10X₄²

Where,
X₁= Enzyme concentration (mg/100g dry matter), X₂ = Incubation time (h), X₃ = Incubation temperature (⁰C) and X₄ = Tempering water pH

The response surface quadratic model optimized the pre-treatment as enzyme concentration 37.80 mg/100 g dry matter, incubation time 8.69 h, incubation temperature 48.48 ⁰C and tempering water pH 5.49 which gave the predicted values of hulling efficiency 88.37 %, protein content 21.57 % and cooking time 21.91 min.

The hulling efficiency, protein content and cooking time of oil treated (control) sample were found 76.25 %, 19.12 % and 26.8 min, respectively while the observed values of hulling efficiency, protein content and cooking time at the optimum conditions of enzymatic pre-treatment variables was 88.12 %, 21.81 % and 21.50 min, respectively. Hence, there was an increase in hulling
efficiency of 13.47 %, Protein content of 12.33 % and decrease in cooking time 19.77 % over oil treated sample.

The predicted values of hulling efficiency, protein content and cooking time obtained from equations showed 0.28, 1.10 and 2.19 % deviation from the experimental values, respectively. It could reveal that the experimental values were very close to the predicted values which confirmed the optimum conditions.

Sensory evaluation indicated that both the treatment, i.e., enzyme and oil treated cooked blended dhal got almost equal ratings in terms of taste and flavour while there was little variation in colour and textural ratings.

From the above study, it could be recommended that better recovery and quality of pigeon pea dhal could be obtained by enzymatic pre-treatment of enzyme concentration of 37.80 mg/100 g dry matter, 8.69 h incubation time, 48.5 °C incubation temperature and 5.49 tempering water pH gave a hulling efficiency 88.12 %, protein content 21.81 % and cooking time 21.50 min. The quantity of enzymes required have been estimated considering the 10.46 % (w.b.) moisture content of pigeon pea normally used by the pulse mills. The suggested method could save time, energy consumption and labour to a great extent and beneficial to the pulse milling industry.
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APPENDIX A

Calculation of weight of enzyme required for preparation of different enzyme concentrations

Enzyme concentration

Sample size = 2 kg

Initial moisture content = 10.46 % (wb)

\[
\text{Amount of water in 2 kg sample} = \frac{\text{Moisture content} \times \text{Weight of sample}}{100} = \frac{10.46 \, \text{(w.b.)} \times 2000 \, (g)}{100} = 209.2 \, \text{g}
\]

Dry weight of sample = 2000-209.2

= 1790.8 g

For enzyme concentration i.e. 20 mg/ 100 g dry sample

Weight of enzyme required for 1790.8 g dry sample

\[
= \frac{20 \times 1790.8}{100} = 358.16 \, \text{mg}
\]

<table>
<thead>
<tr>
<th>Enzyme concentration mg/ 100 g dry sample</th>
<th>Total weight of enzymes (mg)</th>
<th>Enzyme Proportion (2 : 1 : 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Xylanase (mg)</td>
</tr>
<tr>
<td>20</td>
<td>358.16</td>
<td>179.1</td>
</tr>
<tr>
<td>30</td>
<td>537.24</td>
<td>268.6</td>
</tr>
<tr>
<td>40</td>
<td>716.32</td>
<td>358.2</td>
</tr>
<tr>
<td>50</td>
<td>895.40</td>
<td>447.7</td>
</tr>
<tr>
<td>60</td>
<td>1074.48</td>
<td>537.2</td>
</tr>
</tbody>
</table>
# APPENDIX B

Total husk content of pigeon pea grains

<table>
<thead>
<tr>
<th>Replications</th>
<th>Weight of cotyledon (g)</th>
<th>Weight of husk (g)</th>
<th>Total weight (g)</th>
<th>Husk content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.610</td>
<td>1.370</td>
<td>9.98</td>
<td>13.73</td>
</tr>
<tr>
<td>2</td>
<td>8.815</td>
<td>1.335</td>
<td>10.15</td>
<td>13.15</td>
</tr>
<tr>
<td>3</td>
<td>8.466</td>
<td>1.284</td>
<td>9.75</td>
<td>13.17</td>
</tr>
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### APPENDIX C1

**Physical properties of pigeon pea**

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APPENDIX C2

Physical and frictional properties of pigeon pea

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**APPENDIX D**

Weight of different fractions, actual husk removed and hulling efficiency obtained from oil treated (control) samples 

(n=3)

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<th>Husk (g)</th>
<th>Powder (g)</th>
<th>Unhulled grain (g)</th>
<th>Total Weight (g)</th>
<th>Total Husk Content</th>
<th>Actual Husk removed</th>
<th>Ch</th>
<th>Cwk</th>
<th>HuIl. Effi. (%)</th>
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<th>Cooking time (min)</th>
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OT = Oil treatment

Ch = coefficient of hulling

Cwk = Coefficient of wholeness of kernel
APPENDIX E

Weight of different fractions, actual husk removed and hulling efficiency obtained at different combination of variables

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<th>Powder (g)</th>
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Appendix F

Weight of different fractions, actual husk removed and hulling efficiency obtained at optimized level of variables

\[(n=3)\]

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Ch = Coefficient of hulling
Cwk = Coefficient of whleness of kernel