



Role of Enzymes in Pulse Milling

Poonam Choudhary, Surya Tushir, Manju Bala and S.K. Tyagi

*Division of Agriculture Structures & Environmental Control
ICAR-CIPHET, Ludhiana
E-mail: poonam.choudhary98@yahoo.com*

Introduction

Pulses are an important crop in the world belonging to the Leguminosae family. Pulses have been cultivated for millennia and have been essential for human and animal nutrition as well as pulses pull nitrogen from the air into the soil, improving soil fertility and agronomic systems. Pulses are very good and cheap source of proteins and amino acids in the diets for people around the globe, ensuring food security, especially for poor socio-economic and vegetarians group. In addition to this, pulses also contain considerable amount of minerals, vitamins and crude fibre etc. The world production of pulses was 67 MT and India alone contributed 17 MT (FAOSTAT, 2012). The United Nations declared 2016 the International Year of Pulses. India is the largest producer, processor, importer and consumer of pulses in the world.

Pulse milling

Pulses are mostly consumed in the form of dehusked splits, commonly known as dhal. The outer layer of the grain (husk) is attached to the protein and starch bearing cotyledons of the pulse grains. In some grains like pigeonpea, mungbean, beans and urd bean, this bonding is strong due to the presence of a layer of gums in between the husk and the cotyledons. These are known as difficult-to-mill pulses which needs repetitive pre-treatments with multiple passes through the dehulling machine. On the other hand, grains like chickpea, pea, lentil and lathyrus *etc.*, this bonding is comparatively weaker. Such grains can be milled easily and are categorized as easy-to-mill pulses, which needs pre-treatment only once followed by one or two pass through the dehulling machine (Narasimha *et al.*, 2003). This outer husk layer is required to be separated from the cotyledons and subsequently split in two halves before consumed as dhal. The process of removal of husk/hull from the cotyledons is called dehulling and the entire process of dehulling and subsequent splitting of cotyledons, its cleaning, polishing and grading is known as milling. Dehulling improves product appearance, texture, product quality, palatability and digestibility. Dehulling also removes considerable amount of several anti-nutritional factors such as protein inhibitors, amylase inhibitors, phytic acids and tannins. A substantial amount of avoidable loss takes place at different stages of milling. This may vary from 10-15% depending upon the type and quality of grain milled, the process and machinery used for milling and other factors. It is, therefore, important to look at different aspects of pre-treatment so that proper process is used to obtain maximum recovery of good quality dhal from the grain and take corrective measures to reduce milling losses to the minimum.

Pre-milling treatments

Pre-milling treatments are generally employed to loosen the seed coat to remove husk without losing any edible portion such as cotyledon material and germ. These treatments are usually given to pulses to

- a. Loosen the hull
- b. Ease milling
- c. Reduce breakage
- d. Improve the quality of splits.

Mostly pre-milling treatments are developed for pigeon pea. Pulses have a layer of gum between hull and cotyledons which binds them together. These gums are made up from cellulosic microfibril network in which non-starch polysaccharides (NSP) and proteins (Cosgrove, 1997) are embedded. Besides this, a layer of pectic substances, which are made up of 1,4- β -D-galacturonic acid, branched 1,3- β -D-xylogalacturonan, rhamnagalacturonan, and homogalacturonan also present between seed coat and cotyledon (Round *et al.*, 2010) The chemical nature and

quality of gums regulate the power of hull and cotyledon affection, and, therefore plays important role in dehulling (Tiwari and Singh, 2012). Partial hydrolysis of these NSP and/or proteins by enzymatic reactions may facilitate the easy dehulling of legumes (Arora *et al.*, 2007; Verma *et al.*, 1993). Several pre-milling treatments such as water soaking, oil and water application, mixing of sodium bi-carbonate solution and thermal applications are commonly recommended and adopted pre-milling treatments to loosen the bond between hull and cotyledons. These treatments also reduce the breakage and improve the product quality. Pre-dehulling treatments may also involve heat treatment alone or soaking in water, chemical solutions, tempering followed by hot dehulling (Phirke and Bhole, 2000; Phirke *et al.*, 1992; Ramakrishnaiah and Kurien, 1983; Srivastva *et al.*, 1988). The limitations of these treatments were either a shape deformation or poor cooking quality of dehulled splits. These treatments are also labour intensive and time consuming.

Enzymatic pre-treatments

Enzyme is an important class of globular proteins of biological origin that act as biochemical catalyst and speed up the rate of biochemical reaction 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, 2002). Xylanase, cellulose and protease enzymes are used extensively to hydrolyse hemicelluloses (arabinoxylan and xyloglucan) and protein network, respectively, which are present in the mucilage at the interface of hull and cotyledon (Sreerama *et al.*, 2009). However, enzymatic pre-treatment for easy dehulling of pulses is still limited to laboratory stage.

Functions of enzymes

Xylanase (EC 3.2.1.8): Xylanase degrade the linear polysaccharide β -1, 4-xylane 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 xylene into xylooligosachharides. Xylosidase, debranching enzymes (L-arabinofuranosidase and glucuronidase) and esterases (acetylene xylanesterase, feruloyl esterase) allow the complete degradation of the xylooligosachharides to their monomeric constituents (Whitaker *et al.*, 2003).

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

Pectinase (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. Pectinase break down the pectinases break down the pectin to pectinic acid and finally pectic acid (Whitaker *et al.*, 2003).

Table 1: Effect of enzymatic pre-treatment on milling efficiency of pulses

Pulse Crop	Enzyme source	Milling efficiency	References
Pigeon pea	Enzyme from <i>Aspergillus fumigate</i>	Hulling efficiency 88.9% obtained at 26.6% (w.b.) moisture content, 0.08: 260 enzyme protein grain ratio and 46.7 °C incubation temperature for the period of 12.7 h.	Verma, 1991
Pigeon pea	Partial hydrolysis by enzymes	Hulling efficiency 86.74%	Verma <i>et al.</i> , 1993
Pigeon pea	Food grade mixed xylanase and cellulose enzyme	Hulling efficiency 88.93% at an enzyme concentration of 0.08 g protein per 260 g pigeon pea grain	Saxena <i>et al.</i> , 1993

Pulse Crop	Enzyme source	Milling efficiency	References
Green gram, black gram, red gram and horse gram	Xylanase and protease pre-treatments	Xylanase pre- treatment resulted in hulling efficiency 84.4%, 78.4% and 75.7% dehulled grains in horse gram, green gram and black gram, respectively. 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 number of fines.	Sreerama <i>et al.</i> , 2009
Pigeon pea	Xylanase, pectinase and cellulase	Dehulling efficiency, protein content and cooking time 88.12%, 21.81% and 21.50 min, respectively.	Sangani <i>et al.</i> , 2014a
Pigeon pea	Xylanase, pectinase and cellulase	Dal recovery, milling efficiency, protein content and cooking time 76.60%, 96.19%, 18.56% and 23 min, respectively.	Murumkar <i>et al.</i> , 2016

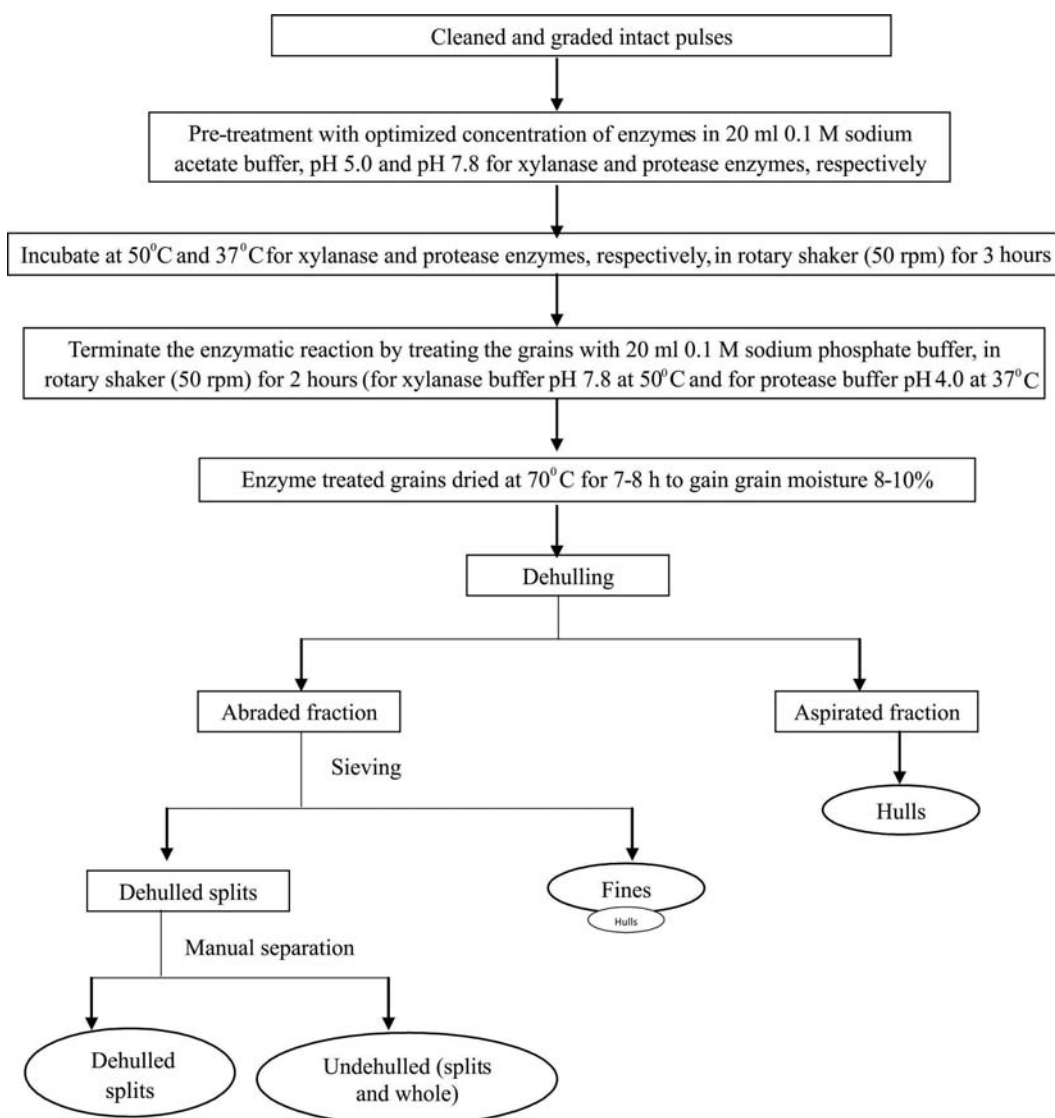


Fig. 1: Flow diagram for pre-dehulling treatment with enzymes and separation of dehulled fractions (Adapted from Sreerama *et al.*, 2009)



Cooking quality

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 (Williams *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. Long cooking time results in a decrease in protein quality and a loss of vitamins and minerals. The cooking time of enzyme (xylanase, pectinase and cellulose) pre-treated pigeon pea dhal was found 21.5 min in comparison to oil treated dhal which required 26.5 min (Sangani *et al.*, 2014b).

Conclusion

Enzyme treated target grains were found to utilize less time for dehulling as compared to water treated grains used in conventional milling. The enzyme treated grains were found to be brighter in colour in comparison to untreated grains. Additionally there were changes observed in the amount of broken grains and powder formation i.e., after processing of the grains, the powder formation and number of broken grains reduced significantly which supports the overall reason for application of enzymes for dehulling. Also due to the application of enzymes, the protein quality or cooking quality were not affected. With all these reasons it can be concluded that, use of enzymes for dehulling improved the overall acceptability and quality of target grains.

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