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Effect of some culture extracts of *Aspergillus oryzae* on dehulling properties of pigeon pea (*Cajanus cajan* L.)

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Pre-dehulling treatments with some culture extracts of *Aspergillus oryzae* of various incubation periods played an important role in improving the dehulling properties of pigeon pea (*Cajanus cajan* L.). Yield percent of dehulled grains and dehulling efficiency increased concurrently with increase of incubation period of culture extracts. Maximum dehulled grains were achieved by 12-day old culture extract to the tune of 73% with least amount of undehulled kernels and fines (6.6 and 6.5%, respectively). Pre-dehulling trials conducted on pigeon pea grain employing wheat bran and pigeon pea husk based culture extracts of *A. oryzae* showed dehulling efficiency of 73% for wheat bran (12-day incubation period) and pigeon pea husk (9-day incubation period) in comparison with uninoculated extract (control) in the range 62.2-64.4%. Based on the results obtained, dehulling properties affected by pigeon pea husk based culture extract proved better than wheat bran culture extract.

Key words: Dehulling, pigeon pea, culture extracts, incubation periods, pre-dehulling treatment.

INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is a tropical grain legume grown mainly in India. The crop represents about 5% of world legume production with more than 70% being produced in India (Odeny, 2007). The high nutritive value of pigeon pea is perhaps the most important reason why it finds an important place among the smallholder poor farmers in India. Pigeon pea is abundant in protein, making it an ideal supplement to traditional cereal-based diets of most Indians which are generally protein-deficient. Generally, pigeon pea contains 20–25% protein and is consumed after suitable processing (Tiwari et al., 2008). Researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India have developed high protein lines (HPL) with up to 32.5% protein content and significantly higher sulphur-containing amino acids (cysteine and methionine) (Singh et al., 1990; Saxena et al., 2002). Pigeon pea is therefore a good source of amino acids (Berrios et al., 1999).

Pigeon pea seeds are mainly eaten as dry decorticated split cotyledons by a milling process called dehulling. Dehulling is defined as the removal of the outer hull (fibrous seed coat or testa) which is tightly attached to the

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cotyledons (Kurien and Ramakrishnaiah, 1985). Dehulling is one of the most important processes applied to pigeon pea and is practiced widely in Asia and Africa on either a home or cottage industry scale (Tiwari et al., 2010). Dehulled seeds take less time to cook and have acceptable appearance, texture, palatability, digestibility and overall nutritional quality. There are two approaches to remove hulls, namely wet and dry milling. Generally, the dry method of milling is used throughout the Indian subcontinent for milling of pigeon pea because the quality of splits obtained from wet milling is poor (Kurien and Parpia, 1968; Kulkarni, 1991). The maximum theoretical recovery of dehulled pigeon pea is around 87-89%, whereas traditional dehulling recovery is only about 65-75% (Singh, 1995). Pigeon pea is recognized as hard-to-dehull pulse because of the presence of strong bond between the hulls and cotyledons usually via a thin layer of gums and mucilages along with uronic acids in the form of calcium pectate (Kurien and Ramakrishnaiah, 1985). Dehulling of pigeon pea incurs loss to the tune of 10 - 12% (broken grain and powder) edible cotyledon invariably (Mangaraj and Singh, 2011). Pre-treatment is required prior to removal of the hull to: (a) loosen the hull, (b) ease milling, (c) reduce breakage and (d) improve the quality of splits (Tiwari et al., 2007). Pigeon pea hulls can be loosened with pre-treatments of oil, heat or chemicals (Saxena et al., 1990). But importantly all these methods confront limitations of shape deformation or poor cooking quality of dehulled split and are time consuming even (Phirke and Bhole, 2000). A novel pre-dehulling technique involving enzyme is prospective to improve dehulling efficiency upon reducing the dehulling loss and improving cooking quality of pigeon pea (Deshpande et al., 2007; Sreerama et al., 2009; Bhowmik, 2012; Sangani et al., 2014). Partial hydrolysis of the mucilaginous bonds (in the interface of hull and cotyledon) by enzymatic reactions facilitates the easy dehulling of legumes (Verma et al., 1993). Enzyme mediated degradation of cell wall polysaccharides of horse gram and pigeon pea resulted in expansion of the grain with improved nutritional and functional properties upon thermal treatment as documented by Sreerama et al. (2008a, b). Enzymes are also reported to be utilized in rice polishing in a more selective way through hydrolysis of cell wall polysaccharides (Arora et al., 2007; Das et al., 2008) and aqueous extraction of vegetable oil by rupturing of oil-seed liposomes (Tano-Debrah and Ohta, 1999; Vierhuis et al., 2001; Lamsal et al., 2006). However, high cost of pure enzyme may be disadvantageous for its commercial application in dehulling venture. One of the alternative approaches to overcome this obstacle is to make continuous search for microorganism(s) and/ or their consortium for production of desired enzyme(s) in copious amounts and to optimize enzyme(s) production with them under submerged culturing condition for desired dehulling effect on application to pulses ahead. Optimization of enzyme production upon manipulation of fungal growth substrate, fermentation period are proven (Narasimha et al., 2006; Acharya et al., 2008). However, research in manipulation of microbial growth substrate and fermentation period for optimization of dehulling properties of pulses is meagre. Generally, hydrolytic enzymes, e.g. cellulases, xylanases, pectinases, etc. are produced by fungal cultures, since such enzymes are used in nature by fungi for their growth. *Trichoderma* spp. and *Aspergillus* spp. have most widely been used for these enzymes. *Aspergillus oryzae,* a multicellular fungus producing multiplicity of hydrolytic enzymes (namely cellulases, *β*-glucosidase, protease, lipase, *α*-galactosidase, *β*-galactosidase, *α*-amylase, glucoamylase etc) on several agro-substrates is well reviewed by Pandey et al. (1999). Enzymes of *A. oryzae* have been used for several years in food processing, feed preparation, waste-water treatment, detergent formulation, textile production and other areas. Moreover, *A. oryzae* is considered to be a safe organism for production of food enzymes because it lacks expressed sequence tags for the genes responsible for aflatoxin production (Machida et al., 2005). In view of biotechnological importance of the *A. oryzae*, the present study emphasizes and evaluates the dehulling properties of pigeon pea using wheat bran and pigeon pea husk based culture extracts of *A. oryzae* developed under different incubation periods in order to optimize enzyme fermentation in different culture extracts for effective dehulling of pigeon pea. The cellulase activity and protein content in the culture media in relation to growth of *A. oryzae* for all treatments under study are also assessed.

MATERIALS AND METHODS

Raw material

Pigeon pea (of a white variety) was purchased from a local market in Ludhiana, India. The grains were thoroughly cleaned and were passed through 5.0 and 4.5 mm round holed sieve. The overflow of 5.0 mm sieve and underflow of 4.5 mm sieve was rejected and grain size between 5.0 and 4.5 mm was used for conducting the experiments.

Population density and culture extract

Aspergillus oryzae a local isolate from ITCC, IARI, New Delhi was grown in submerged culture of wheat bran media (Zambre, 1994). 100 ml of water deionised was amended individually with 4% wheat bran and sterilized (15 psi for 15 min) in Erlenmeyer flask of 500 ml capacity. Pigeon pea husk based media was prepared in similar fashion in parallel. The flasks were inoculated with 1% spore suspension of 6-day old *A. oryzae* grown on Potato Dextrose Agar (PDA) with 2 x 10^6 spores and incubated at 28±2°C on rotary shaker (180 rpm) for 3, 6, 9 and 12 days. Uninoculated media as corresponding controls were however incubated for 3 days only (on obtaining negative microbial growth results in test of sterility in Petri plates). The fungal population density at 3, 6, 9 and 12 days of incubation along with control was examined by most-probable-number (MPN) method upto 1:10^6 dilution in 0.9% (w/v) NaCl water and was expressed as colony-forming units (CFU)/ ml from separate

set (Bhowmik et al., 2013). Flasks were withdrawn at 3 days interval over a period of 12 days and filtered through Whatman No.1 filter paper to separate the mycelial mat of fungi with other coarse particles. The liquid filtrates were centrifuged at 10000 rpm at 10°C for 10 min. The supernatant solutions, thus obtained are natural multi-enzyme laden culture extracts (CEs) of *A. oryzae* of 3, 6, 9 and 12-day old incubation periods. The uninoculated media received similar treatments for control extracts production. These CEs were stored below 4°C and later employed for pre-treatment trials and enzyme analysis tests in this investigation.

Pre-dehulling treatment

CEs of 3, 6, 9 and 12-day old were added to pigeon pea (200 g) individually at optimized ratio of 0.5 (v/w) in sterile 1 L Erlenmeyer flask. The flasks were plugged, shaken manually and equilibrated at 7°C for 8 h. The CE treated kernels were later incubated at optimized conditions of 35°C for 3 h. The optimum ratio, incubation period and temperature were deduced from culture extract-to-pigeon pea ratio, incubation period, incubation temperature in the range of 0.16 - 1.84 (v/w), 0.95 - 11.05 h, 31.6 - 48.4°C (Zambre, 1994; Sarkar et al., 1995; Deshpande et al., 2007; Sreerama et al., 2009) respectively. Finally, the seeds were heated in a recirculatory air drier at 70°C for 15 min to inactivate crude enzymes of CE and dried (50°C for 4-6 h) to minimal moisture of 9-10%. Control seeds were also subjected to similar processing conditions except with extracts of uninoculated media.

Dehulling process

Moisture content of pigeon pea at the time of dehulling was measured according to the method of AACC (1995) and expressed as an average of three determinations. A batch-type laboratory mill (Model No. TM 05 Satake Grain Testing Mill Satake Engineering Co. Ltd, Tokyo, Japan) fitted with abrasive wheel at 4 mm exit clearance was used for dehulling of pigeon pea. The samples (100 g) were dehulled by maintaining abrasive wheel speed 400 rpm for 40 s. After dehulling, hulls were collected by aspiration. The abraded fractions were sieved through 2 mm sieve to collect fines and powder. The material remained in the sieve was manually separated as dehulled and undehulled kernels (Figure 1). All fractions were weighed and then expressed as proportion of the total original sample weight.

Dehulling data analysis

Dehulling index (DI) was calculated using the following equation (Sreerama et al., 2009):

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

where, W_1 is the initial weight of sample taken for dehulling (g), W_2 is the weight of dehulled grains (g), W_3 is the weight of undehulled grains (g), W_h is the weight of hulls (g), W_b is the weight of brokens and powder (g).

The degree of hull removal is the percentage of dehulled kernels to the initial weight of sample taken for dehulling. The degree of dehulling (DD) was defined using the following equation (Sreerama et al., 2009):

$$DD(\%) = \frac{W_1 - W_3}{W_1} \times 100$$

Dehulling efficiency (DE) is an estimate of the efficiency of producing the major product, dehulled kernels. It was calculated using the following equation (Sreerama et al., 2009):

$$DE(\%) = \frac{W_1 - (W_3 + W_h + W_b)}{W_1} \times 100$$

Enzyme assay

Total cellulase activity of inoculated and uninoculated extracts was determined in terms of filter paper units (FPU) (Mandels and Andreotii, 1976). Aliquots of appropriately diluted extracts as enzyme source was added to Whatman No.1 filter paper strip (1 x 6 cm; 50 mg) immersed in one millilitre of 0.05 M sodium citrate buffer of pH 5.0. After incubation at 50°C for 1 h, the reducing sugar released was estimated by dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of filter paper (FPU activity) was defined as the amount of enzyme releasing 1 µmol of reducing sugar from filter paper per ml per min. The content of soluble protein in the extracts was also estimated with bovine serum albumin as a standard (Lowry et al., 1951).

Statistical analysis

The core experiments were repeated three times independently, and the results were represented as mean ±SD. The mean values for population density were transformed to logarithmic form and data were subjected to ANOVA followed by DMRT ($P \leq 0.05$). Correlation analysis between microbial growth and enzymes and proteins production was analyzed using statistical analysis system (SPSS, version 13).

RESULTS

Dehulling properties

The yields of different milled fractions of pigeon pea thus treated with CEs of *A. oryzae* of various incubation periods in the study along with their corresponding controls and their moisture contents at the time of dehulling are shown in Table 1. Increase in yield of percent dehulled grains was directly proportional to the increase of incubation period of CEs. Maximum dehulled grains were achieved by 12-day CE to the tune of 73% with least amount of undehulled kernels and fines (6.6 and 6.5%, respectively). Undehulled grain yield was significantly low ($P \leq 0.05$) with treatments of CE to control samples (with the range of 6.5-9.6% and 15.5-17.7% respectively). Yield of fines and hulls in all treatments are in the range of 6.3-9.3 and 10.9-13.9% respectively. Pigeon pea pre-treated with wheat bran and pigeon pea husk based CEs yielded higher amounts of dehulled kernels (with the range 71-73%) as compared to corresponding control samples (with the range 62-64%) with minimal cotyledon loss (6-8%) during milling. The CE treatments are also statistically significant ($P \leq 0.05$) to the controls. Overall, pigeon pea husk based CE performed better than wheat bran at every instance.

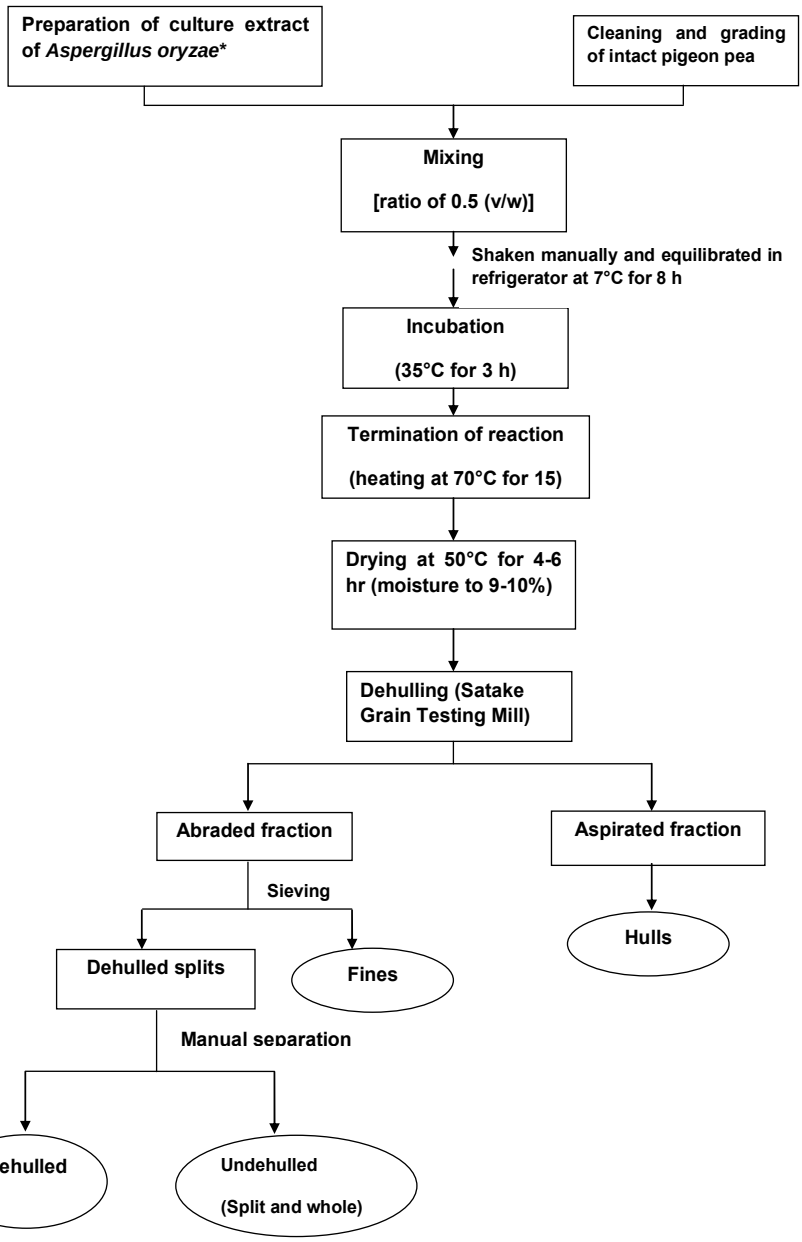

Figure 1. Flow diagram for dehulling and separation of dehulled fractions. *Culture extracts prepared from wheat bran and pigeon pea husk based media.

Table 1. Comparative analysis of the yield of milled fractions of pigeon pea pre-treated with wheat bran and pigeon pea husk based culture extracts of *A. oryzae* of various incubation periods (3-day, 6-day, 9-day, 12-day)*.

Dehulling treatment	Moisture content** (%)	Dehulled grains (%)	Undehulled grains (%)	Fines (%)	Hulls (%)
Wheat bran					
Control	9.7	61.9±0.5^{d}	17.7±0.8^{a}	9.3±0.4^{a}	10.9±0.9^{d}
3-day	9.0	70.8±0.8^{b}	9.6±0.7^{c}	8.0±1.9^{abc}	11.5±0.5^{cd}
6-day	9.0	71.4±0.4^{b}	7.8±0.8^{def}	7.8±0.6^{abc}	12.9±0.6^{ab}
9-day	9.2	70.8±0.5^{b}	8.9±0.8^{cd}	7.1±0.5^{abc}	13.1±0.8^{ab}
12-day	9.9	72.9±0.9^{a}	6.6±0.4^{fg}	6.5±0.6^{bc}	13.9±0.7^{a}

Table 1. Contd

Pigeon pea husk					
Control	9.9	64.0±0.7^{c}	15.5±0.5^{b}	8.8±0.3^{ab}	11.4±0.3^{cd}
3-day	9.0	71.5±0.7^{b}	8.3±0.7^{d}	8.0±2.1^{abc}	12.2±0.7^{bc}
6-day	9.2	71.4±0.4^{b}	7.9±0.9^{de}	7.8±2.1^{abc}	12.8±0.8^{ab}
9-day	9.0	73.1±0.8^{a}	6.5±0.5^{g}	6.3±1.0^{c}	14.0±0.7^{a}
12-day	9.9	72.8±0.2^{a}	6.8±0.5^{efg}	6.5±1.0^{bc}	13.8±0.7^{a}

Mean values bearing different superscript in the same column are significantly different ($P \leq 0.05$) according to Duncan's multiple range test; *Results are mean ± standard deviation of three determinations. **Moisture content (w.b.) at the time of dehulling expressed as an average of three determinations.

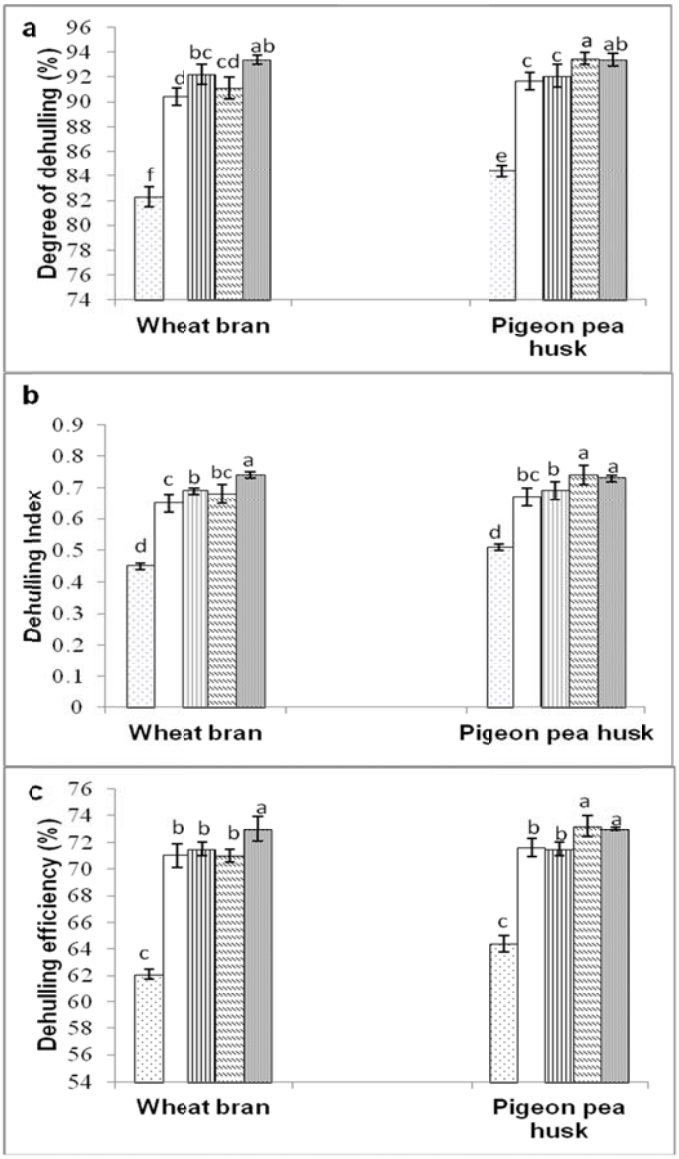

Figure 2. Dehulling properties of pigeon pea pre-treated with wheat bran and pigeon pea husk based culture extracts of *Aspergillus oryzae* of various incubation periods (▯= control, ▯= 3-day, ▯ = 6-day, ▯ = 9-day, ▯= 12-day). Bars represent ± SD of mean (of three replicates). Histograms with a common letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Statistically ($P \leq 0.05$) 9-day old pigeon pea husk based CE is optimum to achieve maximum dehulled grains (73%) with minimum fines (6.3%) among all the treatments under study. The highest DD (93.4%) and DI (0.74) in wheat bran based culture extract were observed in 12-day old CE (Figure 2). Since higher amount of dehulled kernels were obtained in this treatment, the DE (73%) was also significantly higher ($P \leq 0.05$) than corresponding control (62.1%). Statistically significant increase in DD, DI and DE of wheat bran CE treated pigeon pea were observed as compared to corresponding control. However, treatments between different CE incubation periods are not significant at all ages except 12-day at 5% level of probability. Pigeon pea husk based CE treatment also resulted in higher DD as compared to its corresponding control (Figure 2). However, no significant ($P \leq 0.05$) effect among the various ages of pigeon pea husk based CE were recorded. Increase in DD, DI and DE was progressive upto 9-day old CE which declined at the later age. However, the effect of 9-day old pigeon pea husk CE on DD, DI and DE of pigeon pea was at par with 12-day old pigeon pea husk CE at 5% level of probability. Least DD, DI and DE were attained by 3-day old CE (in the range of 90.4-91.7%, 0.65-0.67 and 71-71.6% respectively). Pigeon pea husk based CE recorded maximum dehulling efficiency (73.2%) by 9-day incubation period while wheat bran achieved the maximum (73%) by 12-day incubation period. Never-theless, statistically insignificant effect exists between the treatments of wheat bran and pigeon pea husk CEs.

Fungal growth and cellulase activity

Growth of *A. oryzae* in terms of population density increased with progressive increase of incubation period with a range 7.1-7.7 log CFU/ml (Figure 3). Of the two lignocellulosic agro-wastes in the study, wheat bran supported maximum growth of *A. oryzae.* However, statistically ($P \leq 0.05$) growth was not significant with the increase of incubation period. The cellulase activity in wheat bran and pigeon pea husk media increased concomitantly with the rise in population density of *A. oryzae* as incubation period advanced (Figure 4).

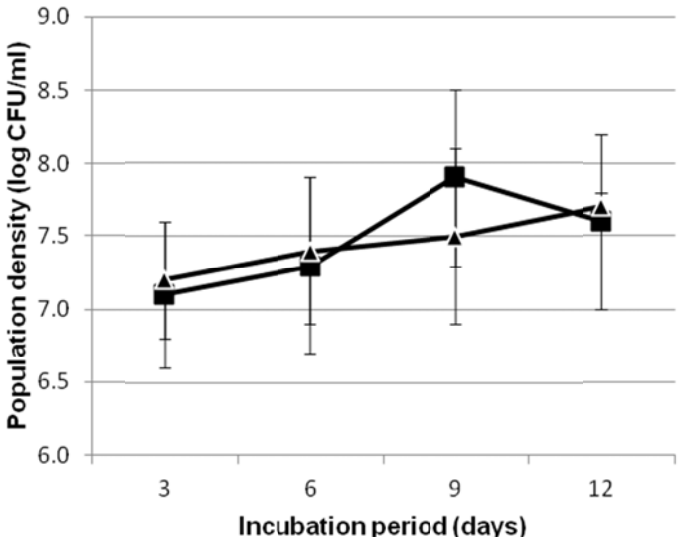

Figure 3. Change of population density of *A. oryzae* in wheat bran (■), and pigeon pea husk (▲) media during different incubation periods. Bars represent ± SD of mean (of three replicates).

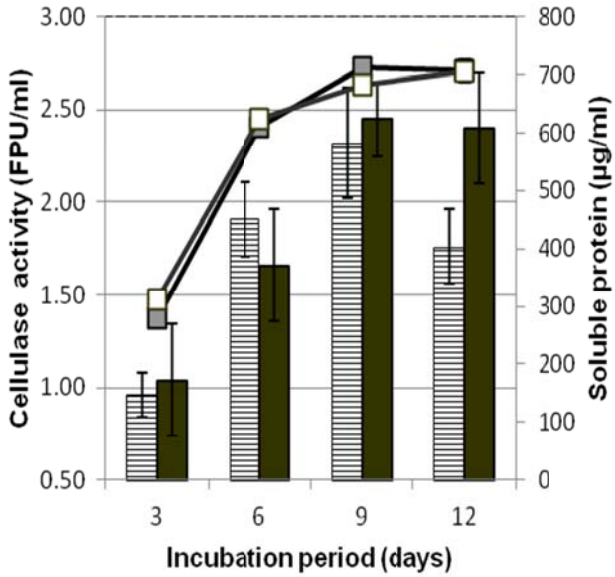

Figure 4. Change of cellulase activity and total protein content during fermentation of *A. oryzae* in wheat bran and pigeon pea husk based media. Histograms represent cellulase activity in wheat bran (▤) and pigeon pea husk (■), line graphs represent total protein content in wheat bran (▩) and pigeon pea husk (□) media, and bars represent ± SD of mean (of three replicates).

Maximum cellulase activity of about 2.3 FPU per millilitre of wheat bran based CE and 2.5 FPU per millilitre of pigeon pea husk based CE was attained at 9-day interval. The extracellular soluble protein content in the CE of two media also increased concomitantly with the incubation period with maximal values ranging from 705-712 µg/ml. No growth, cellulase activity and protein content were detected in both uninoculated media

Table 2. The correlation analysis between *A. oryzae* growth and cellulase and soluble protein production in culture extract.

Parameters	Correlation coefficients
Growth x cellulase	0.882*
Growth x soluble protein	0.875*
Cellulase x soluble protein	0.973**

*Correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed).

(corresponding controls). The correlations between microbial growth at varied incubation period and enzyme production were analyzed (Table 2). For cellulase and soluble protein in CE, correlation coefficient was 0.882 and 0.875, respectively ($P<0.05$). Cellulase activity showed high correlation coefficients to protein content. This was 0.973 ($P<0.01$). The results indicated a significant positive correlation between incubation period and enzyme production in CEs.

DISCUSSION

Strong bonds of gums and mucilage existing between hull and cotyledon are attributed to the hard dehulling nature of pigeon pea (Ramakrishnaiah and Kurien, 1983; Kurien and Ramakrishnaiah, 1985). Several findings in literature reveal the biochemical variations in proportion and structural arrangement of non starch polysaccharides (NSP) and glycoproteins in the seed coat, gums/mucilages and cell wall of legumes (Ramakrishnaiah and Kurien, 1983; Ryden and Selvendran, 1990; Swamy et al., 1991; Showalter, 1993; Stolle-Smits et al., 1995; Cosgrove, 1997; Stolle-Smits et al., 1997; Bravo et al., 1999; Wood et al., 2014). Pre-treatments are generally required to loosen the pulse seed coats including pigeon pea (Singh, 1995). Traditionally vegetable oil treatment (up to 1%) is used in commercial mills in Indian sub continent to loosen the husk of pulses that are difficult to mill (Sokhansanj and Patil, 2003). The recovery of dehulled kernels in commercial mills averages about 75% (Kulkarni, 1989; Kurien, 1981). Comparable yield (73%) of dehulled kernels of pigeon pea by applying CEs of *A. oryzae* was obtained in laboratory process in this study. This finding is also at par with dehulled kernel recovery (71.3 to 73.9%) in pigeon pea when tested with enzyme consortium (Deshpande et al., 2007). However, variable results of pigeon pea dehulled kernel recovery were documented when tested with different enzymes (namely xylanase- 58.9% and protease-78.4%) individually (Sreerama et al., 2009). This may be attributed to the incomplete accessibility of NSP or proteins for hydrolysis by diverse enzymes in isolation due to the nature of substitutes, presence of phenolic compounds such as flavonoids or lignin of lower molecular weight as reported in lima beans and chickpeas (Kannenburg and Allard, 1964; Knights, 1989). However, enzyme consortium enables attaining dehulled kernels to desired level by concerted hydrolytic action (of different enzymes in group) on the complex NSP/proteins in a cascade thus loosening the husk from cotyledon efficiently. CE of *A. oryzae* is a natural milieu of multiple hydrolytic enzymes of commercial value (Pandey et al., 1999). Moreover tempering of the pigeon pea seeds with 0.5 (v/w) with CE for three hours during enzyme reaction has provided a grain moisture content of around 25%. This may have resulted in a moisture gradient within the seed (a relatively high moisture content seed coat as compared to a relatively drier cotyledon). Moisture gradient facilitates the partial hydrolysis of NSP and proteins located in the interface between the seed coat and cotyledon by enzymes (Sreerama et al., 2009). Hence DI value of CE treated pigeon pea (0.74) is higher than the maximum DI value reported for pigeon pea (0.67) with steam treatment at 97°C followed by drying at 120°C (Opoku et al., 2003).

The efficacy of enzymes in dehulling process is a function of moisture content of grain, chemical composition of seed (husk and intermediate gums/mucilages), incubation temperature, incubation period and enzyme concentration (Sangani et al., 2014). But more importantly, it is essential to search enzyme(s)/enzyme consortium with broad spectrum hydrolytic capacity to loosen seed coats of wide variety of pulses having varied cell wall composition genetically. Consideration of universal enzyme consortium in this regard shall not only be unrealistic but also expensive. The only viable option is to search for microorganism(s) producing multiplicity of hydrolytic enzymes suited for pulse dehulling. We selected *A. oryzae* and used its multi-enzyme laden CEs to hydrolyse the NSP/glycoproteins at the interface of hull and cotyledon. Moreover, *A. oryzae* secretes different enzymes (namely cellulases, *β*-glucosidase, protease, lipase, *α*-galactosidase, *β*-galactosidase, *α*-amylase, glucoamylase, etc) at varied proportion in response to the nutrients in its growth medium (Pandey et al., 1999). Optimization of enzyme production by controlling fermentation temperature, pH, nutrients (carbon, nitrogen) and fermentation period is quite achievable. The cellulolytic activity under study is comparable to that of the most well studied fungus, *Trichoderma reesei* whose wild type or mutant cells in free status produced cellulase within a range of 1-2 FPU/ml on various media (Domingues et al., 2000). The production of cellulase by wild type cells of *Bacillus pumilus* (Kotchoni and Shonukan, 2002) and *Cellulomonas biazotea* (Rajoka et al., 1998) and *Trichoderma aureoviride* (Zaldivar et al., 2001) in liquid did not exceed 1.5 U/ml. The overall trend of dehulled kernel yield of pigeon pea under the influence of CEs of *A. oryzae* was 12-day > 9-day> 6-day> 3-day> control. The best period for the production of enzyme by *A. oryzae* was the 12-day (73%), but the 9-day observed a decline in cellular growth. The results suggested that enzyme production was

not growth dependent. This finding is in conformity with the earlier reports (Cho et al., 2002; Purwanto et al., 2009; Darah et al., 2013). Influence of fermentation periods of CEs was positively correlated to DD, DI and DE. However, statistically insignificant (P<0.05) decline occurred after the 9th day in case of pigeon pea husk based CE. Slight fall of hydrolytic enzymes at later growth phase is the plausible reason for the decline in dehulling properties. Insignificant (P<0.05) results within the uninoculated controls in relation to all dehulling parameters were noticed. Dehulled kernel recovery of controls was the least ranging from 62-64%. Absence of enzymes in the control extract is the reason for this effect. This is hence evident from this work that natural enzymes secreted by *A. oryzae* in CE were vital to loosen the husk upon improving the dehulling properties of pigeon pea. Pigeon pea husk based CE was better performer than wheat bran in improving the dehulling properties of pigeon pea which we report for the first time. Relatively high protein (18%) and low cellulose (10%) content in wheat bran to pigeon pea husk (2 and 22% respectively) may have brought variability in production of enzymes in respective CE (Schwarz et al., 1988; Pandey et al., 1999; Prasad et al., 2011). Relatively high cellulase content and comparable protein content of pigeon pea husk to wheat bran is the probable reason for the better performance. This variable nutrients content in the lignocellulosic wastes per se offers a probable scope for harnessing microbial potential to the best in improving the dehulling of pulses.

Conclusion

Low cost enzymes or enzyme consortia are the utmost need when opting for enzyme-assisted pulse milling on commercial scale. Naturally produced multi-enzymes from agro-waste based culture extract of effective *A. oryzae* are advantageous option in this regard. This fungus can produce vital enzymes of commercial value namely cellulases, *β*-glucosidase, protease, lipase, *α*-galactosidase, *β*-galactosidase, *α*-amylase, glucoamylase, etc. The prospects of low cost enzyme is that it can economize the pulse dehulling cost by replacing costly vegetable oil (used traditionally on commercial lines at present) while keeping the natural shape of decorticated split cotyledon of pigeon pea intact. Enzyme-assisted dehulling of pulses may also be potential to improve the cooking quality of pulses which is lacking in oil-assisted pre-milling process. Moreover, the technology of CE can be utilized in both manual and automated dehulling process effectively. The technical drudgery of preparing, mixing and storing of CE is relatively less than pure enzyme in dehulling process. The results indicated that microbial density in progressive incubation period directly correlates to increase in enzyme production and increase of dehulled pigeon pea with reduced undehulled grains and fines subsequently. Here, pigeon pea husk based CE performed well than wheat bran based CE with regards to dehulling properties including dehulling efficiency. It can be summarized from this study that CEs from different lignocellulosic substrates have different potential to influence the dehulling process of pigeon pea. Moreover, the optimum incubation period for enzyme production by *A. oryzae* is a source of activated enzyme to improve dehulling properties of pigeon pea effectively.

Conflict of interest

The author(s) have not declared any conflict of interests.

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