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Research paper

Bioactive compounds of whole, dal, milling byproduct and fractions of milling byproduct of pigeonpea (*Cajanus cajan* L.)

Prasoon Verma, Man Mohan Deo*, Vaibhav Kumar, Krishnashis Das, Manisha Parashar, Deepshikha and Narendra Kumar

ICAR-Indian Institute of Pulses Research, Kanpur - 208 024, Uttar Pradesh, India

* ***E-mail**: mm.deo@icar.gov.in*

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Dr. Narendra Kumar, ICAR-Indian Institute of Pulses Research, Kanpur, India

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ABSTRACT

Milling of pigeonpea is an essential process to improve culinary properties. Byproducts obtained from the milling have many bioactive components which can have significant favorable effects on human health. In commercial mills, pigeonpea dal recovery is about 70% against the potential dal recovery of 85%. As the seed coat is tightly attached to the cotyledons due to the presence of a gum layer in between. During abrasive dehusking, a commonly adopted method in commercial mills, about 15% of protein-rich peripheral cotyledons get mixed with seed coat. The milling byproduct thus obtained includes pulse proteins in the form of broken, powder of cotyledons, and phenol and antioxidant rich husk. The present study focuses on biochemical estimation of pigeonpea whole, dehusked splits (dal), milling byproduct, and fractions of milling byproduct. The husk and the broken fraction of milling byproduct (about 75% of the byproduct) are rich in phenolic compounds, antioxidants, and dietary fiber, whereas the remaining cotyledon powder fraction is found to be rich in protein. These components were separated and analyzed for biochemical estimations. The results indicate that cotyledon powder separated from pigeonpea milling byproduct contains the highest protein (20.6%). Husk fraction of the byproduct is reported to have maximum total phenolic content (845.55 mg GAE/100g) and total antioxidant activity (59.68 m mole TE/100 g). Dehusked splits were observed to have the highest calorific value (386.17 kcal/100g). Till now, the milling byproduct is used as low-value cattle feed. Results of the biochemical studies reveal that protein, phenol and antioxidant-rich pigeonpea milling byproduct can be utilized for the development of value-added edible products and also find applications in therapeutic usage.

Key words: Byproduct, Pulse milling, Total antioxidants, Total phenols, Value-added products

INTRODUCTION

Pigeonpea (*Cajanus cajan* L.), an important food legume of the Indian subcontinent, plays a significant role in human nutrition. The whole grain of pigeonpea comprises 85% cotyledons, 1% of embryos, approximately 14% of the seed coat, and several dietary nutrients (Faris and Singh, 1990). About 90% of the total protein content is present in cotyledons of pigeonpea. Although germ contains 48.1% protein, it is negligible in comparison to whole seed weight (Srivastava and Ali, 2004). In pigeonpea seeds, the major components include protein, carbohydrates, vitamins, and minerals. Whole seeds also contain different antinutritional factors (ANF) but in minor quantity. Some of these antinutritional factors include oligosaccharides (raffinose and verbascose), enzyme inhibitors (trypsin, chymotrypsin, and amylase), polyphenols (phenols and tannins), and phytolectins. Cooking of pigeonpea seeds destroys some ANFs, which improves the bio-availability of various nutrients and also enhances starch digestibility (Salunkhe, 1982). Toms and Western (1971) reported that the seeds are also free of hemagglutinating activity. Duhan *et al*. (2001) reported a significant reduction in anti-nutritional factors of pigeonpea by simple inexpensive cooking methods such as soaking, dehulling, sprouting, and pressure cooking. The dehulling process removes the seed coat, which eliminates some outer layers of cotyledons and protein-rich germ. In commercial milling of pigeonpea, about 70% dal is recovered against the potential dal recovery of 85%. About 15% of precious cotyledons get mixed with the husk and obtained as milling byproduct. In traditional methods of pigeonpea milling, about 55-70% of cotyledons are recovered against the potential cotyledon recovery of 88-89%. This indicates excessive loss of cotyledons in the form of broken, and powder (Sinha *et al*., 2017). The milling byproduct is found to be a rich source of protein and polyphenols, exhibiting an important functional property of antioxidant activity to scavenge various free radicals (Tiwari *et al*., 2013).

Thus, due to its nutritional and functional properties, pigeonpea milling byproducts have tremendous potential to be used as an ingredient in the development of different value-added edible products. The present study was conducted to determine nutritional parameters, viz., protein content, total phenols, total antioxidant activity, and calorific values of pigeonpea whole grain, dal and fractions of milling byproducts. The powder component of milling byproduct can directly be substituted as pulse protein in the diet whereas phenol, antioxidant and fiber-rich husk fraction can find use as functional food with therapeutic advantages.

MATERIALS AND METHODS

Sample preparation

Cleaned and graded pigeonpea (*Cajanus cajan* L.*)* grains were washed and soaked in water for 8 hours. Soaked grains were sun-dried to achieve moisture content up to 10-12% (Wet basis, w.b.) (Patil and Mangraj, 2013) and milled in an abrasive roller dehusking unit of the IIPR Mini Dal mill. As a result, pigeonpea dal and milling byproducts were obtained. The milling byproduct was fractionated with the help of an electromagnetic sieve shaker (Electrolab EMS-8) into two fractions, fraction A: greater than 0.25 mm size (husk and broken), and fraction B: less than 0.25 mm size (powder of cotyledons). Dried whole seeds, dal, byproduct, and their fractions were further converted into powder for biochemical analysis using a laboratory grinder (Perten, Model: Laboratory mill-3303). Then all the resulting 5 samples obtained, viz., whole seeds, dal, byproduct and both fractions (fraction A and fraction B) were subjected to biochemical analysis. The sample preparation process is shown in the flow chart Fig 1.

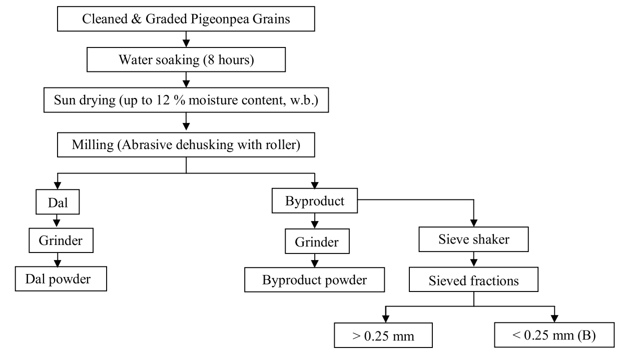

Fig. 1. Preparation of sample

Biochemical analysis of samples

The biochemical analysis of all the samples, viz., whole grain, dal, byproduct, byproduct fractions greater and less than sizes of 0.25 mm (Fig. 2), includes estimation of protein, total phenolic content, total antioxidant activity, and calorific value.

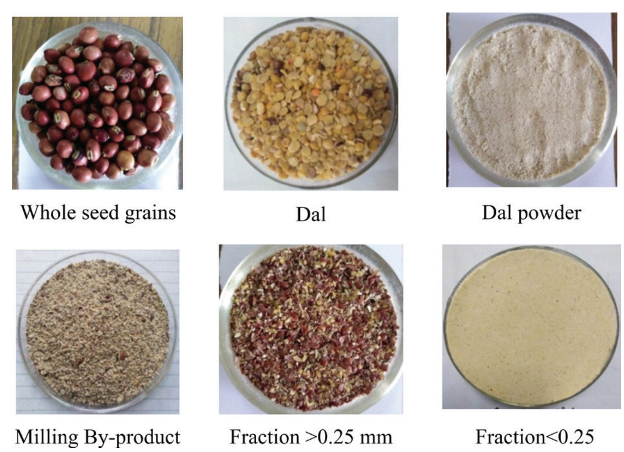

Fig. 2. Whole, dal, and fractions of milling byproduct of pigeonpea

Estimation of protein content

All samples were subjected to quantification of protein by Lowry's method (Lowry *et al*., 1951). The extraction step was slightly modified according to Maehre *et al*. (2016) (salt/ alkaline extraction). The sample (100 mg) was ground in 10 ml of grinding solution (0.1 M NaOH in 3.5% NaCl) followed by incubation in a water bath (60ºC) for 90 min. It was then centrifuged at 6000 rpm for 10 minutes at 4ºC. For quantification, 50 µl of aliquot was allowed to react with copper sulphate and Folin's reagent to obtain blue colored complex. The concentration of protein was determined through a calibration curve against Bovine Serum Albumin (BSA) standard.

Total phenolic content determination:

The total phenolic content (TPC) was estimated by a Folin-Ciocalteu assay (Singleton and Lamuela-Raventos, 1999). The sample (500 mg) was ground in 5 ml of 70% ethyl alcohol. It was then subjected to shaking at 200-300 rpm for 3 hours in an incubator shaker in the dark. After centrifugation at 6000 rpm for 15 minutes at room temperature, the pellet was re-extracted with 5 ml of 70% ethanol by incubation shaking for another 45 minutes, and the supernatant was cooled to room temperature. From this, 200 µl (100 µl for byproduct powder, and fractions A and B) of aliquot was taken in a test tube and 250 µL of 1 N Folin-Ciocalteu's reagent was added. Then 3 ml of double distilled water and 7% $NaCO_3$ (750 µL) was added sequentially. The reaction mixture was subjected to vortexing followed by incubation of 8 min. at room temperature. Then 800 µL of double distilled water was added and after 30 min, the absorbance was measured at 765 nm in a spectrophotometer (Thermo Fischer, Biomate 3). Phenols in the sample were calculated as gallic acid equivalents (mg of GAE /100 g sample) using standard curve of gallic acid.

Total antioxidant activity estimation:

For analyzing the total antioxidant capacity of all the samples, the CUPRAC method was used as described by Apak *et al.* (2008). The sample (200 mg) was soaked overnight in 20 ml of 70% acetone. The content was then centrifuged at 3000 rpm for 10 min and then the supernatant was stored at 4ºC. Take 1 ml each of Neocuproine (2,9-dimethyl-1,10-phenanthroline) alcoholic solution, a copper (II) chloride solution, and an ammonium acetate aqueous buffer at pH 7 in a test tube. To this 100 µl (of whole seeds and fraction B), 50 µl (of byproduct and fraction A and B), and 200 µl (of dal) of sample extract were added to make the final volume up to 4.1 ml. The absorbance was recorded at 450 nm against reagent blank after 30 minutes incubation and antioxidant capacity was expressed as Trolox (6-hydroxy-2,5,7,8- tetramethyl chroman-2-carboxylic acid) equivalent in terms of m mole TE/100 g of seed using the formula,

$$\mu mol\ TE/g = (A_f/€_{TR})\ (V_f/V_s)\ r\ (V_{initial}/m)$$

where, $V_{initial}$= initial volume, m = weight of sample, r = dilution factor, V_f= final volume, V_s= volume of aliquot, A_f= absorbance, $€_{TR}$= 1.67 X 10^4 L $mol^{-1}cm^{-1}$

Determination of calorific value

The calorific value of each component was determined using IKA C200 Bomb Calorimeter. Initially, the calorimeter was calibrated using a 0.5 g Benzoic acid C723 tablet with a known calorific value of 26460 J/g and RSD of 0.03%. After grinding the whole pigeonpea, dal, byproduct, and fractions of byproduct in a laboratory grinder (Perten), the powders were converted into pellets equivalent to benzoic acid tablets and used for calorific value estimation inside a bomb calorimeter. The oxygen pressure inside the bomb was used up to 30 kg/cm^2 to burn the sample placed in the crucible and placing thread properly inside the bomb releasing energy in terms of calories.

All the biochemical analysis was performed in triplicate and data were analyzed using MS-Excel and OPSTAT software.

RESULTS AND DISCUSSION

Protein content

The highest protein content (20.6%) was observed in byproduct fraction B (<0.25 mm) which is rich in cotyledons as the major protein storage part of pulse seed, in comparison to husk fraction containing the least amount of protein, i.e., 14.26%. The protein content of fraction B was followed by cotyledons (Dal), whole seed, byproduct, and fraction A. Their respective values were observed to be 19.39, 18.38, 16.10, and 14.26 percent respectively. There was a significant ($p<0.05$) difference in protein contents of whole seed, dal, byproduct, and byproduct fractions. The variation of total protein content in different fractions is shown in Fig. 3 (a). In the case of pigeonpea, the seed coat is attached more tightly to the cotyledons due to the presence of gums, in comparison to other legumes. This makes dehulling of pigeonpea difficult than other pulses. The multiple passes over abrasive dehusking surfaces resulted in loss of cotyledons and reduction in dal recovery. Cotyledons of seed are rich in carbohydrates (66.7%) and one-third part of the seed coat consists of fiber. The major portion (50%) of seed protein is located in the embryo. Singh and Jambunathan (1982) studied protein concentration in a different part of pigeonpea and analyzed that embryo consist of high albumin protein content than that of other parts of the seed. Reddy *et al.* (1979) reported the outer layer is rich in protein content in comparison to the inner layers of cotyledons. Dehulling of whole grains of pigeonpea removes husk and germ, and outer layers of the cotyledon (rich in protein) are converted into a very fine powder which comes in the fraction B (<0.25 mm) through an electro-magnetic sieve shaker and, thus, fraction B is the richest fraction in protein.

Total phenolic content

The total phenolic content of all the pigeonpea samples was estimated. The husk fraction (Fraction A) contains the highest amount of phenolic components, viz., 845.55 mg GAE/100 g followed by the whole byproduct (Fraction A + B) 763.56 mg GAE/100 g. The cotyledon power (Fraction B) is having phenol content of 239.55 mg GAE/100 g followed by whole grain and dal fractions as 107.77 and 74.95 g GAE/100 g, respectively. The variation in total phenolic content among different fractions of pigeonpea is shown in Fig. 3 (b). Analysis of variance showed that there was a significant difference in total phenolic content of the whole seed, dal, byproducts, and byproducts fractions at a 5% level of significance. It has already been reported that the removal of the seed coat also eliminates the protein-rich germ and some outer layers of cotyledons. Some fine husk fraction also gets mixed with Fraction B of the milling byproduct. Husk component (A) of milling byproduct of pigeonpea processing is considered as a naturally available rich source of polyphenols and some protein due to the presence of broken cotyledons. Thus, this phenol-rich husk component of byproduct with water soluble and insoluble fibers, anti-cancerous and antioxidant substances can also be used in developing various edible value-added products of therapeutic advantages, besides being used as animal feed. Phenols are responsible for colors in food products and are known for antioxidant activity. During dehulling process, the husk contains some portion of the outer layer of cotyledons and some part of the germ, thus, the husk possesses a remarkable portion of free and hydrolyzable phenolic acids, insoluble dietary fiber (Naveena and Bhaskarachary, 2013) as well as some protein also present in this. These polyphenols exhibit various health benefits due to their anti-inflammatory and antioxidant nature (Scalbert *et al.*, 2005). Various experiments conducted on animals and human cells demonstrated that these polyphenols play a significant role in the prevention of cancer and cardiovascular diseases (Singh and Jambunathan, 1982).

Total antioxidant capacity

The antioxidant activity was found in correlation with total phenolic content (Xu and Chang, 2011; Marathe *et al.*, 2011). Thus, the highest antioxidant capacity was observed in husk (Fraction A) as 59.68 mmole TE/100 g followed by milling byproduct and whole grain, viz., 52.45 and 11.07 mmole TE/100 g, respectively. The antioxidant value of fraction B was observed to be 6.69 and the lowest antioxidant value was found in cotyledons as 2.28 mmole TE/100 g. The representation of variation in antioxidant activity in different fractions of pigeonpea is shown in Fig. 3 (c) which is analogous to phenolic content variation in different evaluated fractions. Analysis of variance showed that the total antioxidant capacity of whole seed, dal, byproducts, and byproducts fractions varied significantly at a 5% level of significance. Due to the presence of polyphenols, husks possess the capacity of free radicals scavenging, thus, exhibiting antioxidant activity and preventing free radical-induced damage to bio-molecules like 2-deoxy-D-ribose and hemoglobin (Tiwari *et al.*, 2013).

Calorific value

It was calculated by using a Bomb Calorimeter and presented as kcal/100 g of seed. The total amount of energy released was found highest in cotyledons followed by whole seed and byproduct viz., 386.17, 377.20, and 352.87 kcal/100 g, respectively. The lowest content of calories was found in powder of milling byproduct (Fraction B), i.e., 330.92 kcal/ 100 g and for husk (Fraction A) 341.74 kcal/100 g was observed to be slightly higher than cotyledon powder. The variations in the amount of energy

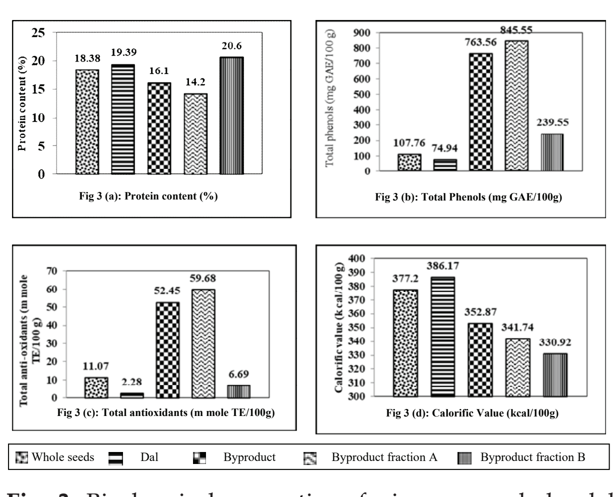

Fig. 3. Biochemical properties of pigeonpea whole, dal and byproduct fractions

released by different fractions of pigeonpea are presented in Fig. 3 (d). Analysis of variance for calorific value of whole seed, dal, byproducts, and byproducts fractions showed significant difference at a 5% level of significance. It has been reported that the protein content, total phenols, and total antioxidant activity of whole seeds lie within the range, i.e., 18-25%, 38.6-542.7 mg GAE/100 g and 3.5-11.08 mmole TE/100 g, respectively (Parikh *et al*., 2018), that also matches with the observation reported in the present study.

CONCLUSION

The biochemical information of pigeonpea milling byproduct indicates that a fraction greater than 0.25 mm in size (Fraction A) can be utilized for developing value-added products rich in phenol and antioxidants. The food value of the milling byproduct less than 0.25 mm (Fraction B) is equivalent to that of *dal* in terms of proteins. Pulses protein of this fraction can directly be used for human consumption and the development of pulse-based home recipes and commercial products. Edible use of pulse milling byproducts will further be explored for various ready-to-eat or cook kinds of value-added edible products. The fractions of pigeonpea milling byproduct also possess various therapeutic properties, thus, can be used as nutraceuticals, antioxidants, cholesterol-lowering fibers, and anti-cancerous edible products. Formulations of food products from such an inexpensive source like the milling byproduct of pigeonpea offer a useful alternative strategy to combat malnutrition and increase the availability of pulses protein for the vegetarian population.

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