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Biochemical Compositions of Milling Byproduct of Mungbean and its Fractions

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ABSTRACT

Background: Mungbean is consumed as whole, sprouts or dehusked splits, in form of dal. Dal is prepared after removal of outer husk cover and splitting the cotyledons in abrasive emery roller mills to improve esculent properties. In the process of husk removal, milling byproduct, mixture of husk and cotyledon powder, is generated, which is usually utilized as low value cattle feed. The milling byproduct contains bioactive compounds useful for human health. In the study biochemical properties of two mungbean cultivars, namely, Shikha and Virat, were evaluated for whole, dal milling byproduct and its fractions, to explore using milling byproduct of mungbean for potential edible usages.

Methods: In this study, mungbean cultivars were milled in abrasive roller after water soaking pre-milling treatment. Milling byproduct was fractionated into three particle sizes, i) >1.0 mm, ii) >0.125 mm and iii) <0.125 mm. Biochemical components, *viz*, protein, phenols, antioxidant activity and calorific values were estimated for whole grain, dehusked split (Dal), milling byproduct and its fractions for the two selected mungbean cultivars.

Result: The protein content was observed to be high in split dal and powder fraction (<0.125 mm) of milling byproduct. Antioxidant value and phenolic compound were higher in the byproduct fraction retained over > 1.00 mm sieve size due to presence of husk in this fraction. Calorific value of byproduct was higher than that of whole seed, dal and byproduct fractions. The study indicates that the mungbean milling byproduct, with beneficial bioactive component can be utilize to develop value added edible products of therapeutic and health benefits.

Key words: Antioxidant activity, Calorific value, Dehusking, Milling byproduct, Phenolic compounds.

INTRODUCTION

Mungbean is also known as greengram, believed to be Indian originated crop and it is widely cultivated in Asian countries like Thailand, Burma, Indonesia, Philippines. Now it is also widely cultivated legumes in Africa, South America, Australia and United States (Blessing and Gregory, 2010). Mungbean is a source of high-quality protein, complex carbohydrates (dietary fibre), minerals and vitamins. It possesses many bioactive compounds *viz.* polyphenols, flavonoids and antioxidants which are responsible for reducing risk of coronary heart diseases, diabetes, obesity and lowers blood cholesterol level. It is generally consumed as whole, sprouts and dehusked splits (dal). Mungbean are digested very easily and have low borborygmus, that is why physicians generally suggest to take mungbean dal to patients. It is rich in phosphorus and provitamin A and free from antinutritional factors comparatively other legumes (Naik *et al.* 2020, Nazir *et al.* 2018). Mungbean contain carbohydrate (51%), protein (24-26%), mineral (4%), vitamins (3%) and fat (1%). Mungbean seeds consist mainly three parts, namely, testa, *i.e.*, seed coat (12.2-23.5%), cotyledons, *i.e.*, splits dal (76.5-87.2%) and embryo, *i.e.*, germ (2-3%). Cotyledons are foremost rich in protein. Mungbean also contains minerals such as phosphorus (P), calcium (Ca) and iron (Fe). Calcium is principally present in the seed coat (30-50%), iron in the embryo (23 mg/100 g dry weight) and phosphorus in cotyledons (341 mg/100 g dry weight) (Haryanto *et al.* 2020, Anwar *et al.* 2007).

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Dehusking and splitting of mungbean two cultivars, Shikha and Virat, were done in emery roller unit of IIPR Dal Mill after water soaking as pre-milling treatment. The milling byproduct generated during milling were fractionated and analysed for protein, phenols, antioxidant and calorific values along with that of whole grain and dal. Possibilities had been explored to utilize mungbean milling byproduct to develop nutritionally rich edible products from it.

MATERIALS AND METHODS

Sample preparation

Mungbean

Whole grains of mungbean were cleaned, graded, washed and dried before pitting in first pass through emery roller unit of IIPR Mini Dal Mill. Pitted grains were soaked in water

for two hours and sundried till 10-12% moisture content. Water soaking pre-milling treatment is prevalent in cottage scale pulse milling. Thus, treated mungbean grains were again milled in the abrasive dehusking unit of the mill (II Pass) to obtain dehusked and split munbean dal. Milling byproduct was collected and used for biochemical evaluation. Electro-magnetic sieve shaker (Electrolab EMS-8) was used to fractionate the milling byproduct into particle sizes >1.00 mm, >0.125 mm and <0.125 mm. Dried whole seeds, dal, byproduct and its fractions were further converted into powder form using laboratory grinder (Pertan) for biochemical analysis. The whole, dal, byproduct and byproduct fractions are shown in Fig 1.

Dal and fraction recovery

Recovery of dal, byproduct and its fractions was determined by calculating weight of the product/fraction obtained against the total sample weight. The observations were converted into percentage. Percentage recovery of all the samples was determined by using following formula.

$$\text{Per cent recovery} = \frac{\text{Obtained sample}}{\text{Total samples}} \times 100$$

Biochemical analysis of samples

The biochemical analysis, *viz.*, protein content, total phenolic content and total antioxidant activity of whole grain, dal, byproduct and fractions of byproduct had been performed in the biochemistry laboratory ICAR-IIPR Kanpur. Percent recovery and calorific values, of different byproduct fractions were determined in the food processing laboratory of the institute.

Determination of protein content

Protein content of products and byproducts was determined using Lowry's method (Lowry *et al.* 1951) and according to Maehre *et al.* 2016 (salt/alkaline extraction) slightly modification had been made in the sample extraction process, in which 100 mg of each sample were grinded in 10 ml of grinding solution (0.1 M NaOH in 3.5% NaCl), and after grinding, samples were incubated in water bath at 60°C for 90 min. After incubation of the sample, it was taken out from the water bath and cooled at room temperature. After cooling, it was centrifuged at 6000 rpm at 4°C for 10 minutes and collect the supernatant in another centrifuge tube. In a test tube, 50 µl aliquot was taken for each sample and 1 ml volume was making up by adding 950 µl distilled water to prepare a reaction mixture. Now, mixture was allowed to react with 5ml solution of 2% Na_2CO_3 in 0.1 N NaOH, copper sulphate, sodium potassium tartrate and vertexing it. After 10 min 500 µl Folin's reagent was added into the reaction mixture to obtained blue colour complex and vertexing it. After 30 min protein concentration was measured by taking OD against blank reagent at 660 nm in UV Spectrophotometer (Shimadzu). The concentration of protein present into each sample was determined by a calibration curve against BSA standard.

Total phenolic content determination

The total phenolic content (TPC) of the samples was determined by a Folin-Ciocalteu assay (Singleton and Lamuela-Raventos 1999). In this process, 500 mg of each sample were grinded in 5 ml of 70% ethyl alcohol and it was kept for shaking at 200-300 rpm for 3 hours in incubator shaker preferably in the dark. After that it was centrifuged at 6000 rpm for 15 minutes at room temperature, and supernatant was separated into another centrifuge tube and pellet was re-extracted by adding 5 ml of 70% ethanol, kept for shaking again for 45 minutes and then supernatant was collected in the same supernatant collected tube and store it at 4°C. In a test tube,100 µl aliquot was taken for reaction mixture and added 250 µl of 1 N Folin-Ciocalteu's reagent. After that 3 ml of double distilled water and 750 µl of 7% $NaCO_3$ was added sequentially. The reaction mixture was vertexing after incubation of 8 min. at room temperature and

Fig 1: Mungbean whole, dal, byproduct and fractions of milling byproduct.

then, 900 µl of double distilled water was added for making of volume 5 ml reaction mixture and after 30 minutes, OD was taken at 765 nm in spectrophotometer. Phenolic content of the sample was calculated as gallic acid equivalents (mg of GAE /100 g sample) using standard curve of gallic acid.

Total antioxidant activity estimation

CUPRAC method (Apak *et al.* 2007) was used for evaluation of total antioxidant activity of all the samples. In this method, 200 mg (powder form) of each sample was soaked in 20 ml of 70% acetone overnight. Soaked sample was centrifuged at 6000 rpm for 10 min at room temperature. Separate the supernatant into another centrifuge tube and store it at 4°C for further analysis. For reaction mixture, take 1 ml Neocuproine (2,9-dimethyl-1,10-phenanthroline) alcoholic solution, 1 ml copper (II) chloride solution and 1ml of ammonium acetate aqueous buffer at pH 7 in a test tube. Then 100 µl of sample extract was added to it and make final volume up to 4.1 ml by adding 1ml double distilled water followed by vertexing. After 30 m minutes incubation, OD of each sample was recorded at 450 nm against reagent blank. Antioxidant activity of the sample were expressed as Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) equivalent in terms of m mole TE/100 gm of sample 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.
$€_{TR}$ = 1.67×10^4 $Lmol^{-1}$ cm^{-1}

Determination of calorific value

IKA C200 Bomb Calorimeter was used for the estimation of calorific value of each sample of mungbean. Initially bomb calorimeter was calibrated using 0.5 gm Benzoic acid C723 tablet with known calorific value as 26460 J/g and RSD 0.03%. For sample preparation, whole pulses, dal, byproduct and fractions of byproduct were grinded in powder form and converted it into pellets of approximately equivalent to benzoic acid tablet. This pellet was kept into crucible inside the bomb calorimeter to determine calorific value of the sample. Then oxygen gas was filled into the bomb up to 30 kg/cm^2 to burn the pellet placed inside the crucible through thread which was touched the sample and energy was released in terms of calories after complete burning of the pellet inside the bomb.

RESULTS AND DISCUSSION

Dal and byproduct recovery

Milling of the pulses was done in emery roller unit of IIPR Mini Dal Mill to obtain recovery of dal and milling byproduct. Dal recovery of mungbean cultivar, namely, Shikha and Virat, were observed as 76.73% and 73.60% respectively, whereas 20.66% and 21.13% byproduct was recovered. Then by product of the two cultivars were fractionated by using electromagnetic sieve shaker and observed 9.57% and 11.64% husk and broken was retained in the above 1mm sieve size, respectively, mainly husk fraction. 66.51% and 68.41% byproduct passes through 1mm size and collected over sieve >0.125 mm sieve for the two cultivars respectively. These two fractions contain broken of cotyledons too. The smallest size byproduct fraction passes through 0.125 mm sieve and 23.92% and 19.94% powder fraction was collected in bottom pan respectively. Upper two fractions mainly contain husk and broken, rich in fiber, phenols and antioxidants, with potential to be utilized as nutraceuticals. The finest fraction are cotyledon powder, which is rich in proteins equivalent to cotyledon powder in terms of nutritive terms and can be used as ingredient to traditional recipes. All fractions of byproduct either can be used separately or together in certain ratios for edible purpose. Recoveries from milling of mungbean from the two cultivars are shown in Table 1.

Table 1: Biochemical components of mungbean (Shikha and Virat).

Mungbean	Protein content (%)	Phenol content (m mol TE/100 g)	Antioxidant activity (mg GAE/100 g)	Calorific value (kcal/100 g)	Recovery (%)
i) Shikha					
Whole seed	21.05 (0.68)	372.26 (10.34)	10.24 (1.48)	289.86	-
Dal	20.57 (0.96)	89.86 (7.99)	2.12 (0.57)	365.36	76.73
Byproduct	21.10 (0.40)	2305.59 (82.10)	51.30 (5.71)	384.16	20.66
>1.00 mm	16.88 (0.66)	2779.30 (156.69)	107.35 (16.53)	334.36	9.57
> 0.125 mm	20.95 (1.26)	2547.41 (41.37)	96.09 (16.07)	376.92	66.51
<0.125 mm	21.71 (1.01)	891.10 (40.47)	29.18 (1.17)	344.80	23.92
ii) Virat					
Whole seed	23.76 (0.28)	328.99 (7.52)	14.43 (0.70)	355.33	-
Dal	23.68 (0.16)	123.10 (3.10)	4.19 (1.41)	356.69	73.60
Byproduct	20.53 (0.61)	1808.37 (46.47)	90.70 (11.63)	352.66	21.13
>1.00 mm	16.11 (0.28)	2669.15 (109.71)	132.80 (22.56)	260.82	11.64
> 0.125 mm	20.00 (0.14)	1556.59 (15.04)	72.70 (4.07)	267.65	68.41
<0.125 mm	18.29 (0.12)	924.65 (26.25)	58.75 (2.65)	320.63	19.94

Protein content

The protein content of selected two cultivars of mungbean, *viz.*, Shikha and Virat are reported in Table 1. The whole seed contained 21.05% and 23.76% protein, whereas dehusked splits (dal) were observed to be 20.57% and 23.68% protein respectively. The average value of protein content for whole grains and splits were evaluated and reported as 22.40% and 22.13% respectively. Average value of overall protein content of milling byproducts of two cultivars were estimated and reported as 20.81%. Fractionated byproduct of the two cultivars has the lowest protein content in upper fraction of sieve due to presence of husk mainly. Protein content of this husk fraction is 16.49% due to cotyledon broken trapped with husk. Middle fractions (>0.125 mm sieve size) and lower fraction (<0.125 mm sieve size) of the byproduct had approximately similar protein content because both fractions were contained cotyledon broken and powder. The protein contents of these fractions were observed to be 20.48% and 20.00%, respectively. Thus, these fractions rich in protein content and similar to cotyledon in nutrient value so that it can be used for edible purpose. Protein contents of dal and powder component of milling byproduct were compared using t-test and protein content in dal was observed to be significantly higher than the byproduct powder <0.125 mm at $p \leq 0.05$.

Total phenolic content

The total phenolic content (TPC) of whole grain, dal, byproduct and fractions of milling byproduct for the two variety *i.e.*, Shikha and Virat of mungbean were estimated. TPC of whole grains were evaluated and reported in Table 1. as 372.26 and 328.99 mg GAE/100 g for the two cultivars, respectively, with an average value of 350.62 mg GAE/100 g. Dal (splits) obtained after dehusking of mungbean was observed the lowest TPC of 89.86 and 123.10 mg GAE/100 g for the two cultivars respectively. This is mainly due reduction of husk part on split cotyledons. TPC of byproduct of the two cultivars was estimated and reported as 2305.59 and 1808.37 mg GAE/100 g respectively. The upper fraction (>1.00 mm sieve size) of the byproduct of both varieties are rich in husk, possessed the highest phenolic content and mentioned as 2779.30 and 2669.15 mg GAE/100 g respectively, with an average value 2724.22 mg GAE/100 g. Middle fractions (>0.125 mm) also rich in phenolic content due to presence of husk fraction *viz.*, 2547.41 and 1556.59 mg GAE/100 g (Average 2052.00 mg GAE/100 g) of two cultivars respectively. TPC of the lower fraction (<0.125) were observed to be 891.10 and 924.65 mg GAE/100 g, with an average value of 907.87 mg GAE/100 g. Whole mungbean contained highest TPC ranged between 38.6-542.7 mg GAE/100 g and splits (without husk dal) contained lowest TPC

Table 2: Average value of biochemical components of mugbean cultivars.

Mungbean	Protein content (%)	Phenol content (m mol TE/100 g)	Antioxidant activity (mg GAE/100 g)	Calorific value (kcal/100 g)	Recovery (%)
Whole seed	22.40	12.34	350.62	322.60	-
Dal	22.13	3.16	106.48	361.03	75.17
Byproduct	20.81	71.00	2056.98	368.41	20.90
>1.00 mm	16.49	120.07	2724.22	297.59	10.61
>0.125 mm	20.48	84.40	2052.00	322.29	67.46
<0.125mm	20.00	43.96	907.87	332.71	21.93

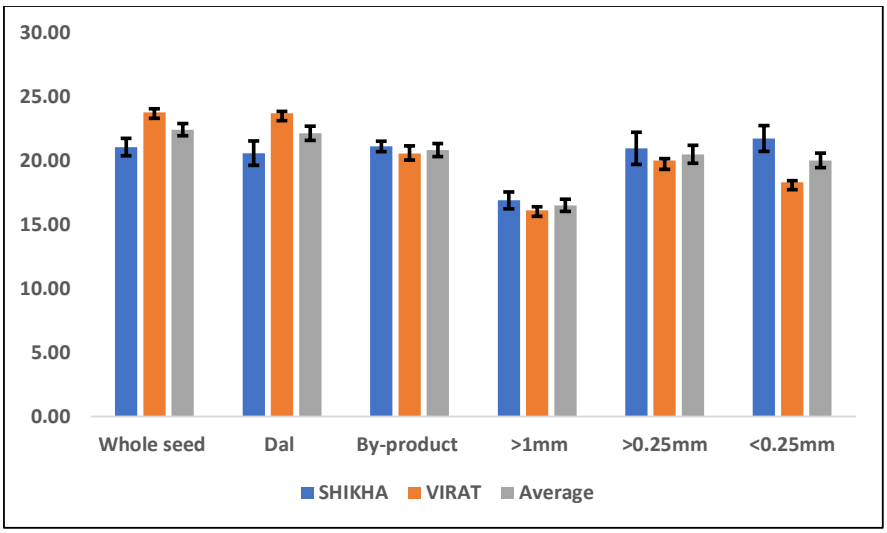

Fig 2: Protein content (%) of mungbean whole seed, dal, byproduct and its fractions of Shikha, Virat and their average.

ranged from 55.2-62.4 mg GAE/100 g (Parikh and Patel, 2018). This showed that TPC of whole grain and dal of our study has approximately fallen within the range. Total phenolic content of whole grain and milling byproduct fraction >0.125 mm was compared using t-test and the difference was highly significant at $p \leq 0.05$.

Total antioxidant capacity

The total antioxidant activity for the whole seeds of mungbean cultivar Shikha and Virat was estimated as 10.24 and 14.43 mmole TE/100 gm, respectively, with an average value of 12.34 mmole TE/100 gm. After dehusking of grains antioxidant value was reduced in the splits (dal) and reported to be as 2.12 and 4.19 mmole TE/100 gm, respectively. Antioxidant activity of milling byproduct of two cultivars were observed and reported in Table 1 as 51.30 and 90.70 mmole TE/100 gm respectively, with an average value 71.00 mmole TE/100 gm. Fractions of byproduct (>1 mm sieve size) possesses the highest amount of antioxidant activity, *viz.*, 107.35 and 132.80 mmole TE/100 gm (average 120.07 mmole TE/100 gm) due to presence of husk in the fraction. Byproduct fraction >0.125 mm sieve size also has higher antioxidant value, because it also contains husk portion, but comparatively less than that retained over >1 mm sieve and value were observed to be 96.09 and 72.70 mmole TE/100 gm for Shikha and Virat cultivars, respectively. Average value of both varieties was reported to be 84.40 mmole TE/100 gm. Similarly, smaller size fraction, *i.e.*, <0.125 mm sieve size contain small amount of husk with cotyledon powder of same particle size. This fraction has antioxidant value lower than the above two fractions, namely >1.00 mm and >0.125 mm. The results are shown in Table 1 as 29.18 and 58.75 mmole TE/100 gm for the two cultivars, respectively. Average value of this fraction was 43.96 mmole TE/100 gm. Thus, lower (<0.125 mm) and middle fractions (<1mm and >0.125 mm) of the byproduct can be utilized for making food

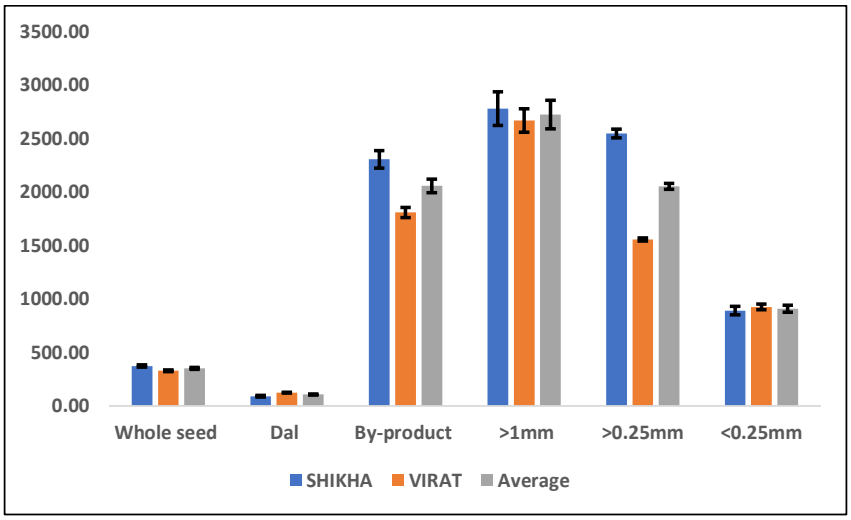

Fig 3: Phenol content (mg GAE/100 g) of mungbean whole seed, dal, byproduct and its fractions of Shikha, Virat and their average value.

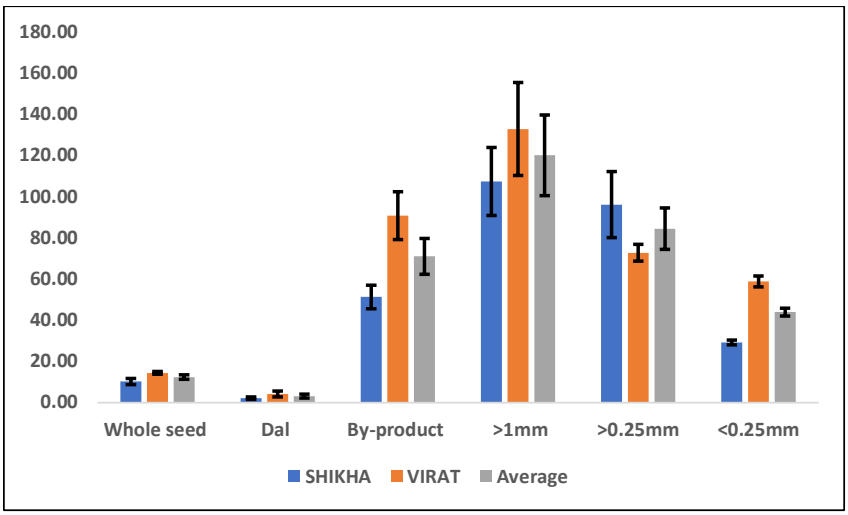

Fig 4: Antioxidant activity (m mol TE/100 gm) of mungbean whole seed, dal, byproduct and its fractions of Shikha, Virat and their average value.

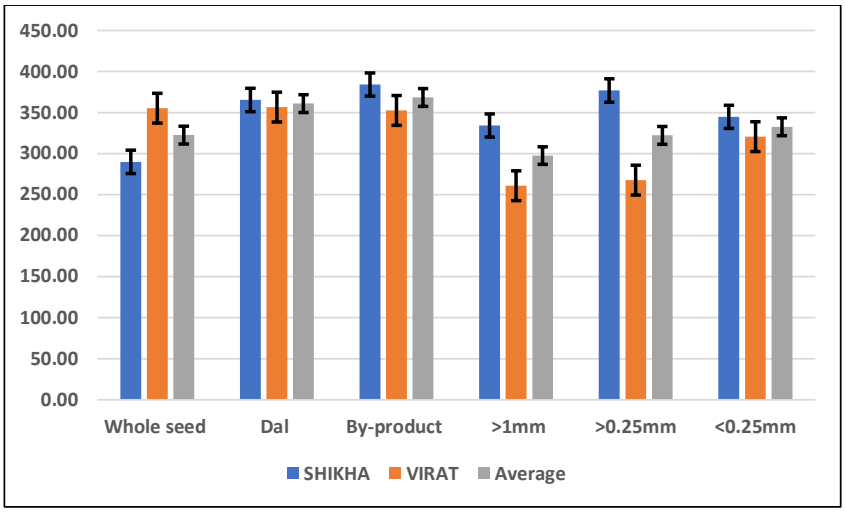

Fig 5: Calorific vlue (kcal/100 gm) of mungbean whole seed, dal, byproduct and fractions of Shikha, Virat and their average.

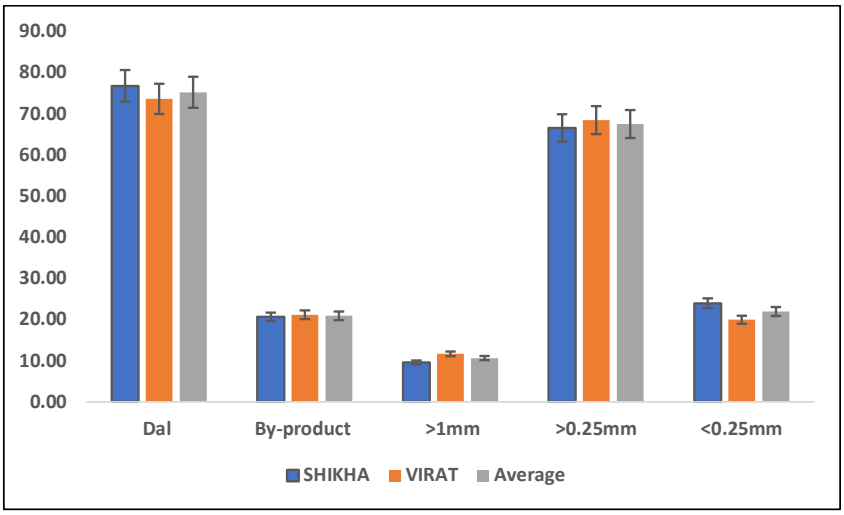

Fig 6: Recovery (%) of mungbean dal, byproducts and its fractions, of Shikha, Virat and their average.

products, rich in protein, antioxidant and polyphenolic compounds. There is significant difference between antioxidant activity of milling byproduct fraction >0.1 mm and that of whole grain at $p \leq 0.05$. High correlations between phenolic compositions and antioxidant activities was observed in of legume, thus, this information can be utilized in developing nutraceutical and food products as legumes have been identified as rich source of antioxidants (Xu and Chang, 2007).

Calorific value

Calorific value of both varieties of mungbean are approximately similar in whole grain, dal, byproducts and byproduct fractions (>1 mm, >0.125 mm, <0.125 mm sieve sizes) was estimated. The average values are reported in Table 2 and observed to be 322.60, 361.03, 368.41, 297.59 322.29 and 332.71 kcal/100gm respectively. For the husk fraction >1 mm, calorific value was observed to be 297.59 kcal/100 gm comparatively lower than that of whole grain, dal, byproduct and fractions. The graphical representation of the different parameters are shown in Fig 2 to 6.

CONCLUSION

The biochemical estimation of mungbean milling byproducts shows that >1 mm size fraction is rich in phenol (2724.22 mg GAE/100 gm), antioxidant (120.07 m mol TE/100 gm) and fiber due to high husk content. Thus, this fraction can be utilized as nutraceuticals and formulated fiber rich value-added products, which have tremendous benefit on human health. Byproduct fraction retained over sieve 0.125 mm sieve and passed through it, contained husk as well as broken or powder of cotyledons are approximately same so that these two fractions can be used for making edible food products *viz*., biscuit, cookies, bakery products *etc*. Smaller size fractions of byproduct *i.e*., <0.125 mm sieve size contained cotyledon in powder form more, so that this protein rich fraction can be utilized in making pulse-based products and traditional recipes. It can be mixed directly whole wheat flour, gram flour and white flour. This fraction can be used for development of protein rich commercial products, *viz*., protein shake, protein soup *etc*. Thus, all the fractions of

mungbean milling byproduct have various therapeutic properties, so there is possibility to use this component as nutraceuticals, antioxidants, cholesterol lowering and anti-cancerous edible products. Development of edible food products by using waste material such as milling byproduct of mungbean and its fractions, can play a major role in combating malnutrition by making more pulse protein available for human consumption specifically for vegetarian population. It can also find application in ready-to-eat or cook fast foods like bread, buns, pizza, noodles *etc*., often considered as junk food, by adding into white flour (maida) for modified food habits of new generation and overcome nutritional deficiency. Powder component of milling byproduct, *i.e*., <0.125 mm can directly be used as pulse protein in the diet whereas husk fraction, rich in phenol, antioxidant and fibre can be used as functional food with therapeutic advantages.

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Conflict of Interest: None.

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