Effect of Water Treatment and Size Reduction on Dietary Fiber Content of Blackgram Husk

Y. Yesritha¹, R. Jaganmohan¹, A. Surendra Babu¹,²

ABSTRACT
The black gram husk a by-product of black gram milling was collected and proceed to washing. The washed and unwashed samples were separated based on the required particle size (900µm, 450µm). The samples obtained were analyzed for their proximate composition and dietary fiber content. The washed husk samples had shown least amount of fat and ash content ranging from 0.53 to 0.77% and 1.04 to 2.47% respectively. The protein content of the samples ranged from 14.43 to 18.65%. The crude fiber content was found to be highest in WBH ranging from 39.9 to 42.13% due to the removal of tailing starches and other cotedelous material. It was observed that the 450WBH sample had the highest amount of Total dietary fiber (TDF), Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF) content. The TDF content of 450WBH was 90.19%. The obtained data was analyzed by paired comparison (t-Test).

Key words: Black gram husk, Fibre, IDF, Milling, SDF, TDF.

INTRODUCTION
Black gram was a pulse crop produced and consumed in South India was considered as a major source of vegetarian protein. Black gram was mainly used in the form of split dhal for consumption which was produced by milling in dhal mills. Milling was a process of dehusking/ dehulling where the seed coat was removed by a series of steps producing abundant amount of by-products like broken, husk, powder etc., accounts for up to 25% of whole grains (Girish, 2012 and Ankit Patras, 2011), that has certain nutritional significance whereas the fruit and vegetable processing would generate 18% of by-products only (Shalini Gaur Rudra, 2015). To facilitate efficient utilization of such by-products the current study was focused on the nutritional significance of black gram husk produced from black gram milling for human consumption. According to Ankit Patras (2011) a 2.5 million tonnes per annum production of pulse by-products was seen in India. Utilizing this by product could benefit both environmentally and economically. The husk produced was utilized as a feed source for animals hence this study was focused on its possible utility as a source of dietary fiber for human consumption as health promoting functional ingredient (Jood, 2014).

Fiber was defined as a mixture of polymeric non starch polysaccharides such as cellulose hemicelluloses and pectin which were resistant to digestion by enzymes in the gastro-intestinal tract (Dhingra et al, 2011). Based on solubility the fibers were classified as soluble and insoluble dietary fibers which had respective health benefits. According to Arulnathan et al. (2013) black gram husk has a significant amount of fiber in the husk/chunies comprising of NDF (48%), ADF (37.41%), hemicelluloses (10.80%), cellulose (26.57%) and lignin (9.64%) respectively. Hence the present study was focused on both soluble and insoluble dietary fiber content of black gram husk. The ratio of soluble to insoluble fiber, particle size and source of fiber were some important attributes for both functional and dietary properties

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(David D, 2003). A ratio of 1:2 of soluble to insoluble fiber was considered acceptable for fibers destined for food use as an ingredient. The soluble to insoluble ratio of bambara groundnut was 1:3 (Maphosa, 2015).

MATERIALS AND METHODS
The unwashed black gram husk sample was obtained from Thangamayil dhal mill at Alangudi, Pudukkottai District, Tamil Nadu. Total Dietary Fiber Assay kit containing Thermostable α-amylose (3000U/mL), Protease (~350 tyrosine U/mL), amyloglucosidase (200U/mL) was obtained from Megazyme Ltd, Ireland. All the other chemicals and reagents were of analytical grade obtained from Sigma Aldrich, Germany.

Pre-Treatment of black gram husk
The black gram husk obtained was washed in batch process according to Nymboire (2012); 500g of sample was mixed with 5L of water and mixed with an electronic mixer at 200rpm for 30min to wash away the foreign material, dust and sand.

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After mixing the husk slurry was drained off water using a muslin cloth. The husk retained on cloth was rinsed in water for the second time to remove any possible fine dust. Both washed and unwashed bran was spread on (72cm×46cm×1.5cm) (l×b×h cm³) stainless steel trays for drying in a tray dryer and dried overnight at 60°C. The samples were named after the pre-treatment as washed black gram husk (WBH) and unwashed black gram husk (UBH). The dried samples were collected in air tight polyethylene covers and stored at 4°C until further study.

Grading of the samples based on particle size
The UBH and WBH samples were graded through 900µm and 450µm sieves and were further named as:
- Unwashed black gram husk with 450 µm particle size: UBH450
- Unwashed black gram husk with 900 µm particle size: UBH900
- Washed black gram husk with 450 µm particle size: WBH450
- Washed black gram husk with 900 µm particle size: WBH900
The samples were packed and stored at 4°C.

Proximate analysis of black gram husk

Determination of moisture content
A sample of 1.5g was weighed into a dry crucible and dried at 105°C for 6h weights were obtained continuously until dried until Equilibrium Moisture Content (EMC). The crucibles were cooled and weights were noted.

\[
\text{Moisture (\%) = } \frac{W1 - W2}{W} \times 100
\]

Where W1 =Initial weight of crucible + Sample, W2 =Final weight of the crucible+ Sample

Determination of ash content
The samples were weighed (W1) into a dried crucible and ignited in a muffle furnace at 550°C for 4h. The final weights (W2) were noted after cooling down in a desiccator.

\[
\text{Ash (\%) = } \frac{W1 - W2}{W} \times 100
\]

Determination of crude protein content
A 1g of samples was weighed into the digestion tube along with 5g Na₂SO₄, 1g , CuSO₄ and 10mL of Conc. H₂SO₄. The tubes were set into the heater of the equipment and digested until completion of the process. These were further distilled with 40% NaOH, 4% Boric acid. The distillate was collected in a conical flask and titrated against 0.1N HCl with mixed with 40% NaOH, 4% Boric acid. The distillate was collected until completion of the process. These were further distillated in air tight polyethylene covers and stored at 4°C until further study.

Grading of the samples based on particle size
The UBH and WBH samples were graded through 900µm and 450µm sieves and were further named as:
- Unwashed black gram husk with 450 µm particle size: UBH450
- Unwashed black gram husk with 900 µm particle size: UBH900
- Washed black gram husk with 450 µm particle size: WBH450
- Washed black gram husk with 900 µm particle size: WBH900
The samples were packed and stored at 4°C.

Proximate analysis of black gram husk

Determination of moisture content
A sample of 1.5g was weighed into a dry crucible and dried at 105°C for 6h weights were obtained continuously until dried until Equilibrium Moisture Content (EMC). The crucibles were cooled and weights were noted.

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\[
\text{Crude Protein (\%) = 6.25* } \frac{N \times (S-B) \times 0.014 \times D}{V \times W} \times 100
\]

Where S =Sample titration reading, B=Blank titration reading, N=Normality of HCl, D=Dilution of sample digestion, V=Volume taken for distillation, 0.014=Milli equilant wt of nitrogen.

Determination of fat content
1g of sample was weighed into filter paper and the thimbles were setup onto the Soxhlet apparatus. Solvent used in the dry extraction method was hexane. After the process the thimbles were dried for 2h at 105°C and the final weights were obtained as the weight of extract:

\[
\text{Crude Fat (\%) = } \frac{\text{Weight of hexane extract}}{\text{Weight of sample}} \times 100
\]

Determination of crude fiber content
According to AOAC (2000, 920.165), 1.6g of sample was boiled for 30min with 1.25% H₂SO₄ and 28% KOH and the filtered residue was washed with 1% H₂SO₄ and NaOH respectively followed by drying for 2h. The dried samples were ignited for half an hour and the final weight of the sample was noted.

\[
\text{Crude fibre (\%) = } \frac{M2-M1}{W} \times 100
\]

Where M1=Mass of the crucible + wet residue weight, M2=Mass of crucible, W =Sample weight

Determination of dietary fiber content
A modification of AOAC method 991.43, Dhingra, D. (2011) was used to determine the TDF, IDF and SDF content of WBH450, WBH900, UBH450, UBH900 samples of black gram husk. One gram of sample was mixed with 40mL of MES-TRIS buffer at 8.2 pH. The solution was homogenized on a magnetic stirrer and 50µL of thermostable α-amylase (3000U/mL) was added and incubated in stirring waterbath for 30min at 100°C. The solution was cooled to 60°C and 100 µL protease (~350 tyrosine U/mL) was added to the solution without pH adjustment and incubated for 30min. pH of the solution was adjusted to 4.5 and 200 µL of amyloglucosidase (200U/mL) was added and incubated at 60°C for 30min. The solution obtained was cooled and filtered using a buchners apparatus. The residue obtained was washed twice with distilled water, ethanol (78%) and acetone (95%). The filtrate obtained was analyzed for the protein and ash content after drying overnight at 103°C. The filtrate was precipitated with four volumes of 95% ethanol at 60°C for 1h. The precipitate was centrifuged to obtain the soluble dietary fiber (Maphosa, 2015).

\[
\text{DF % = } \frac{R1+R2}{\frac{M1+M2}{2}} \times 100
\]

Where R1 = residue weight 1 from m1, R2 = residue weight 2 from m2, m1 = sample weight 1, m2 = sample weight 2, A = ash weight from r, p = protein weight from r, B = blank

Statistical analysis
All the data obtained were subjected to paired comparison test (t- Test) using MS Excel (incl, USA, 2010).
RESULTS AND DISCUSSION

Effect of particle size on the proximate composition of black gram husk

The black gram husk collected from a dhal mill at Tamil Nadu was sieved to obtain 900 and 450 µm particle sizes of both washed and unwashed samples. The values were as follows:

| Component       | Washed husk (WBH) | Unwashed husk sample (UBH) |
|-----------------|-------------------|----------------------------|
| Moisture Content| 10.0±0.0          | 7.30±1.15*                 |
| Crude Fiber     | 42.13±0.12        | 35.63±0.58*                |
| Ash             | 0.77±0.12         | 3.13±0.12*                 |
| Crude Fat       | 1.04±0.07         | 2.13±0.12*                 |
| Crude Protein   | 16.79±0.66        | 18.65±0.78*                |

* significant, ns- not significant

From Table 2 the proximate composition of washed and unwashed black gram husk at 450 µm particle size. Similar to the husk samples at 900 µm particle size a difference was found only in the ash, fat, protein and crude fiber content. Ash, fat and protein content were 0.53% and 2.53%, 2.47% and 3.77%, 14.43% and 18.65% which were in range to the values obtained by (Soris, 2010). The protein content was less than 24.13%, similar to 900 µm particle size. The difference in crude fiber content was about 25.17% which was very much higher than that at 900 µm particle size which might be due to the ease passage of the powdered cotyledon material and germ grits during sifting into the unwashed husk.

Similarly the proximate composition difference between the washed and unwashed black gram husk was given in Table 3 and 4 respectively. The moisture fat and protein content had shown a significant difference from each other due to the removal of other components like starch and germ residues which could account upto 40% according to Ankit Patras (2011). By washing the husk we had obtained a better yield of crude fiber i.e., an increase of 6.5% of crude fiber was seen at 900 µm particle size of black gram husk.

The results of proximate composition for 900 µm particle size sample were presented in Table 1. Washing did not affect the moisture content significantly. The ash, fat and protein content were affected by washing showing a variation of 0.77% and 3.15%, 1.04% and 2.13%, 16.79% and 18.65% respectively similar to the values obtained by Arulnathan et al, (2013). A notable difference was observed in crude fiber content 42.13% and 35.63% in washed and unwashed husk which was close to the 39% fiber content of flaxseed (Nandi, 2015). This might be due to the removal of other components like starch and germ residues which could account up to 40% according to Ankit Patras (2011).

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Table 1: The proximate composition of 900 µm particle size black gram husk.

| Component       | Washed husk sample (WBH) | Unwashed husk sample (UBH) |
|-----------------|--------------------------|----------------------------|
| Moisture Content| 10.0±0.0                 | 7.30±1.15*                 |
| Crude Fiber     | 42.13±0.12               | 35.63±0.58*                |
| Ash             | 0.77±0.12                | 3.13±0.12*                 |
| Crude Fat       | 1.04±0.07                | 2.13±0.12*                 |
| Crude Protein   | 16.79±0.66               | 18.65±0.78*                |

* significant, ns- not significant

Table 2: The proximate composition of 450 µm particle size husk samples.

| Component       | Washed husk sample (WBH) | Unwashed husk sample (UBH) |
|-----------------|--------------------------|----------------------------|
| Moisture Content| 5.7±0.58                 | 5.3±1.15*                  |
| Crude Fiber     | 39.90±0.01               | 14.73±0.12*                |
| Ash             | 0.53±0.12                | 2.53±0.15*                 |
| Crude Fat       | 2.47±0.25                | 2.77±0.15*                 |
| Crude Protein   | 14.43±0.95               | 18.65±0.78*                |

* significant, ns- not significant

Table 3: The proximate composition of WBH.

| Component       | 900µm        | 450µm        |
|-----------------|--------------|--------------|
| Moisture Content| 10±0.00      | 5.7±0.58*    |
| Crude Fiber     | 42.13±0.12   | 39.90±0.01*  |
| Ash             | 0.77±0.12    | 0.53±0.12*   |
| Crude Fat       | 1.04±0.07    | 2.47±0.25*   |
| Crude Protein   | 16.79±0.66   | 14.43±0.95*  |

* significant, ns- not significant

Table 4: The proximate composition of UBH.

| Component       | 900µm        | 450µm        |
|-----------------|--------------|--------------|
| Moisture Content| 7.3±1.15     | 5.3±1.15*    |
| Crude Fiber     | 35.63±0.58   | 14.73±0.12*  |
| Ash             | 3.13±0.12    | 2.53±0.15*   |
| Crude Fat       | 2.13±0.12    | 3.77±0.15*   |
| Crude Protein   | 18.65±0.78   | 14.4±0.95*   |

* significant, ns- not significant

Table 5: The dietary fiber composition of 900 µm particle size BHS.

| Dietary fiber component | WBH         | UBH         |
|-------------------------|-------------|-------------|
| TDF                     | 78.4±0.25   | 71.27±0.08* |
| IDF                     | 75.2±0.05   | 68.3±0.20*  |
| SDF                     | 3.2±0.20    | 3±0.10*     |

* significant, ns- not significant

due to the removal of germ, plumule, aleurone layers C.M. Ajila, U. P. (2009), before the enzymatic digestion of the samples. At 450 µm particle size from Table 6 a difference in TDF, IDF and SDF was seen. Highest TDF was found in the 450WBH which was 90.19% of the total sample with 4.87% of SDF content which was in accordance with the
effect in the yield of fiber, protein and fat content. Variations in the particle sizes of the husk samples before and after the washing procedure had also shown a significant difference in the proximate values of crude fiber, ash and crude fat content. All the results had shown that optimizing the processing parameters and controlling processing steps could lead to a better utilization of milling by-products.

**Table 6:** Dietary fiber composition of 450µm particle size BHS.

| Dietary fiber component | Sample          |
|------------------------|-----------------|
|                        | WBH             |
|                        | UBH             |
| TDF                    | 90.19±0.15      |
|                        | 61.23±0.70*     |
| IDF                    | 85.15±0.05      |
|                        | 60.5±0.04*      |
| SDF                    | 4.87±0.12       |
|                        | 1.37±0.12*      |

* significant

**Table 7:** Dietary fiber composition of WBH.

| Dietary fiber component | Sample          |
|------------------------|-----------------|
|                        | 900 µm          |
|                        | 450 µm          |
| TDF                    | 78.4±0.25       |
|                        | 90.19±0.15*     |
| IDF                    | 75.2±0.05       |
|                        | 85.15±0.05*     |
| SDF                    | 3.2±0.20        |
|                        | 4.87±0.12*      |

* significant

**Table 8:** Dietary fiber composition of UBH.

| Dietary fiber component | Sample          |
|------------------------|-----------------|
|                        | 900 µm          |
|                        | 450 µm          |
| TDF                    | 71.27±0.08      |
|                        | 61.23±0.70*     |
| IDF                    | 68.3±0.20       |
|                        | 60.5±0.04*      |
| SDF                    | 3±0.10          |
|                        | 1.37±0.12*      |

* significant

estimate 87% dietary fiber content in lentils Ankit Patras (2011), while the least percentage of SDF was seen in 450UBHS indicating most of the components were digested by the enzymes during the extraction procedure.

Table 7 and 8 show the values of dietary fiber content in the washed and unwashed black gram husk. From Table 7 the dietary fiber content of washed black gram husk showing a significant difference in the TDF, IDF and SDF content were noted which shows a maximum 90.19% and 4.87% of IDF and SDF content indicating most of the material showing resistance to the digestion (Dhingra 2011). A difference of 12.3% of TDF was seen between the two particle sizes of the washed samples indicating 450WBH having better yield after the extraction.

Table 8 was showing the dietary fiber content of unwashed samples with 900UBHS having the highest TDF content of 71.27% while 78.5% of dietary fiber was seen in the seed coat of black gram husk (Girish 2012) which was lesser than that of 450WBH. The SDF content was 3% which was the highest yield among the unwashed samples. The highest dietary fiber content among the unwashed samples was seen in 900UBH unlike the washed samples which shows that the components of 450UBH were more susceptible to enzymatic digestion.

**CONCLUSION**

From the current study it has been observed that there was a significant difference in the proximate values of black gram husk due to the variations in particle size and washing of the husk. The analysis has shown that washing of the husk obtained from milling unit could produce a significant

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