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Characterization of Colored Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) Varieties for Nutritional, Anti-Nutritional and Nutraceutical Properties

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ABSTRACT

Pulses enhance the protein content of cereal-based diets and improve the composition of the cereal-based diets. Horse gram [Macrotyloma uniflorum (Lam.) Verdc.] is one of the underutilized pulses which is generally called as protein-rich poor man's crop which has a good nutritive profile and resistance to various abiotic and biotic stresses. Three different genotypes of horse gram with different colors viz., black, cream and brown were procured from the University of Agricultural Sciences, Dharwad during the kharif season of 2022 and research on nutrient composition, mineral composition, nutraceutical content, and antinutrient content of horse gram was carried out at Department of Food Science and Nutrition, College of Community Science, Dharwad, Karnataka. Among the proximates, the protein and crude fiber contents were high in brown followed by black and cream-colored variety. The calcium, iron, copper and zinc content were significantly higher in black colored variety followed by the brown and cream-colored variety whereas the manganese content was significantly higher in the black and brown-colored variety. The polyphenol, tannin and antioxidant activity of black-colored variety were higher compared to brown and cream-colored varieties. The soluble, insoluble and total soluble fiber content in colored varieties ranged from 1 to 1.03 per cent, 27.70 to 27.74 per cent and 28.70 to 28.77 per cent respectively. A high amount of antinutrients i.e., phytic acid and oxalic acid was found in black colored variety followed by the brown and cream-colored variety.

Keywords: *Horse gram varieties, Nutrient composition, Mineral composition, Nutraceutical content Antinutrient content, Proximates*

1. INTRODUCTION

India is the largest producer, consumer, and importer of pulses in the world as they are consumed regularly in every household at least with one meal. They enhance the protein content of cereal-based diets and improve the composition of the cereal-based diets. Pulses play a significant role in mitigating the protein malnutrition of millions of poor vegetarians in tropical and sub-tropical regions of the world. Hence, legumes are popularly referred to as "poor man's meat".

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] is one of the underutilized pulses which is generally called as protein-rich poor man's crop that has the ability to grow well even under arid to semi-arid conditions (low rainfall) and marginal soil fertility [1]. U.S. National Academy of Sciences [2] identified horse gram as a potential future food crop because of its good nutritive profile and resistance to various abiotic and biotic stresses. It is considered as super food as it has possessed several medicinal properties since ancient times. Horse gram is used to treat several ailments including diabetes, cancer, kidney stones, menstrual problems, obesity, heart diseases, coughs, colds, digestive problems, and neurological problems [3]. The major drawback that makes horse gram an underutilized pulse is due to its poor amenability to genetic amelioration, poor cooking quality and presence of anti-nutritional factors such as phytic acid and oxalates that hinders the absorption of several important minerals in the human body resulting in poor food quality [4]. These anti-nutrients can be thus reduced by employing several household processing methods such as soaking, boiling, pressure cooking, germinating, roasting, etc. thereby improving the nutritional quality and increasing the bioavailability of other nutrients. Horse gram acts as a potent source of antioxidant along with phenolic content. The defence mechanism in the human body is monitored by the presence of antioxidants which have protective action against the harmful free radicals generated during oxidative stress. Antioxidants also play an important role in inhibiting lipid peroxidation, thus improving food quality and safety [5].

Hence the present study was undertaken to study the nutritional, anti-nutritional and antioxidant properties of colored horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) varieties

2. MATERIAL AND METHODS

2.1 Procurement of raw material

Three different genotypes of horse gram with different colors *viz.,* black (CRHG-22), cream (kalaghatagi local) and brown (GPM-6) (Fig. 1) were procured from Seed Unit, University of

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Agricultural Sciences, Dharwad during the kharif season of 2022 and research was carried out at Department of Food Science and Nutrition, College of Community Science, Dharwad, Karnataka, India

2.2 Proximate composition of horse gram varieties

Proximates including moisture, crude fat, crude protein, crude fiber, ash and carbohydrate content were estimated for all the three colored horse gram varieties

Moisture

A known quantity of sample was weighed into previously weighed moisture cups and dried in hot air oven at 100°C ± 2 °C till constant weight was obtained [6]. Before weighing the moisture cups, each time they were cooled in a desiccator. Moisture content was expressed in per cent and calculated using the following formula,

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

Crude fat

Crude fat was estimated with the principle of crude ether extraction of the dry material. The moisture-free sample (2 g) was accurately weighed and packed in small pouches of Whatman No. 1 filter paper. The pouches were then inserted into a thimble. Then the thimble was placed in a soxhlet apparatus (Socs Plus- Pelican) and extracted with petroleum ether (60 – 80 °C) for two hr. Then, was evaporated after extraction and the flask with residue was dried in a hot air oven at 80-100°C, finally cooled in desiccators, and weighed [6]. The total crude fat was expressed in per cent and calculated by using the formula:

$$\text{Crude fat (\%)} = \frac{\text{Final weight of beaker (g)} - \text{Initial weight of beaker (g)}}{\text{Weight of the sample (g)}} \times 100$$

Crude protein

The protein present in the sample was converted to inorganic nitrogen and distilled and trapped in boric acid, which was titrated against dilute hydrochloric acid. The nitrogen obtained was multiplied with a factor of 6.25 to get the protein content in the sample. The crude protein was estimated in Kelplus instrument of Pelican and crude protein was expressed in per cent [6].

$$\text{Crude protein (\%)} = \frac{\text{(Titre value} - \text{Blank)} \ \text{Normality of HCl} \ 14.007 \ 6.25}{\text{Weight of the sample (g)}} 100$$

Crude fiber

Moisture and fat-free samples were used for estimating crude fiber. The sample was digested with the 1.25 % sulphuric acid and 1.25 % sodium hydroxide solution and the residue obtained was dried in the crucible. The residue was ashed and weighed again. The difference in weight of crucible obtained before and after ignition represents the weight of the crude fiber [6].

$$\text{Crude fiber (\%)} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample (g)}} 100$$

Where W_1 = weight of the crucible (g)

W_2 = weight of residue after drying (g)

W_3 = weight of residue after ignition (g)

Ash

The ash content was determined by the weight loss that occurred during the incineration of 5g of the sample at a high temperature to remove all organic matter from the sample. The sample was heated in a crucible on a heating mantle till all the fumes were emitted and the sample was decarbonized. Then the crucible was ignited in a muffle furnace for 6 hr at 600°C. Further, the crucible was cooled in desiccators and weighed once again [6]. The total ash content was expressed in per cent and calculated by:

$$\text{Total ash (\%)} = \frac{\text{Weight of the crucible after ignition (g)} - \text{weight of the crucible (g)}}{\text{Weight of the sample}} 100$$

Total carbohydrate by difference method

The carbohydrate content was calculated by deducting the sum of the value of moisture, protein, fat, crude fiber and ash from 100 [6].

Available Carbohydrate by difference method

The carbohydrate content was calculated by deducting the sum of the value of moisture, protein, fat and ash from 100 [6].

Energy value

Energy value was computed using Atwater factors of protein, fat and carbohydrate

% Energy = (Protein × 4) + (Carbohydrate × 4) + (Fat × 9)

2.3 Mineral Estimation

Minerals like calcium, iron, zinc, copper and manganese were estimated by digestion using a triacid mixture [6]. A known aliquot of the test sample was suitably diluted and micronutrients like Ca, Fe, Zn, Cu and Mn were determined using an Atomic Absorption Spectrophotometer (Model: ACS- 6880). Calibration of measurement was performed using commercial standard

2.4 Neutraceutical components and antioxidant activity of horse gram varieties

Polyphenols

Total polyphenols were estimated using the Folin-Ciocalteau Reagent. The blue-colored complex was formed in the alkaline medium when phenols reacted with phosphomolybdic acid in the Folin-Ciocalteau Reagent. This blue color complex was measured at 650 nm [7].

Tannins

One ml of methanolic extract was taken in a 100 ml volumetric flask to which five 5 ml of Folin Denis Reagent (FDR) and 10 ml of sodium carbonate solution were added. The contents were mixed and diluted to 100 ml using distilled water and allowed to stand for 30 min and the absorbance was measured at 710 nm. The tannin content of the sample was calculated as tannic acid equivalents from a standard graph [8].

Antioxidant activity

The method used for estimating antioxidant activity was the DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging method. It is an antioxidant assay based on electron-transfer that produces a violet solution in methanol. One g of the defatted sample was refluxed for half an hour using 10 ml methanol, the extract was centrifuged and the supernatant was used for estimation.

One ml of extract was mixed with 0.05 mg/ml of DPPH mixed well and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517nm [9].

Dietary fiber

The soluble, insoluble and dietary fiber fractions were analyzed by enzymatic method [10]. The defatted flour was gelatinized; protein and starch were removed by enzymatic digestion. The residue was filtered washed and quantified gravimetrically for insoluble fiber. The soluble fiber was estimated from the filtrate obtained after enzymatic digestion of protein and carbohydrate of defatted food which is precipitated and estimated gravimetrically. The soluble and insoluble content was calculated. Total dietary is the sum of insoluble and soluble dietary fiber.

2.5 Antinutrient factors in horse gram varieties

Phytic acid

Phytate was estimated by the method described by [11]. The phytate is extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate is determined colorimetrically and phytate phosphorus content is calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate.

Oxalic acid

Oxalates in the samples were estimated by the method given by [12]. In this method oxalates as oxalic acid are extracted from the sample and precipitated as calcium oxalate which is then titrated against standard potassium permanganate.

Statistical analysis: The experimental results were carried out by different statistical methods in SPSS statistical packages (16.0). Mean and standard deviation were used to interpret data. One-way ANOVA (Analysis of variance) was used to know the significant difference among the different colored varieties

3. RESULTS AND DISCUSSION

3.1 Proximate composition of colored horse gram varieties

The proximate composition of colored horse gram varieties is depicted in Table 1. There was a significant difference ($p < 0.01$) between the colored horse gram varieties for moisture, fat, protein, crude fiber, ash, total carbohydrate, available carbohydrate and energy content. The moisture content of cream-colored variety was high (9.2 %) followed by black (8.7 %) and brown (8.31 %) colored variety. When compared to control the moisture content was high for the other two varieties. The fat content of cream and brown-colored varieties was high (0.79 and 0.76 %) followed by the black (0.68 %) colored variety. The fat content of cream colored variety was on par with brown colored variety (control) whereas less for black colored variety. The protein and crude fiber content was high in brown (24.5 and 6.11 %) followed by black and cream-colored variety (22.5 %, 5.85 %, and 21.3 %, 5.29 %) respectively. When compared to control the protein and crude fiber content was less for the other two varieties. The ash content of black-colored variety was high (3.33 %) followed by brown (3.24 %) and cream (3.18 %) colored variety respectively. When compared to control the ash content was high for black whereas less for cream colored variety. The total and available carbohydrate content was high in cream (60.22 and 65.51%) followed by black and cream-colored varieties (58.92 %, 64.78 %, and 57.07 %, 63.19 %) respectively.

When compared to the control the total and available carbohydrate content high for other two varieties. The energy content of the cream and brown colored variety was high (333 Kcal) followed by the black (332 Kcal) colored variety. When compared to the control the energy content was on par for cream colored variety whereas less for black colored variety. _The variations in the proximate composition may be attributed to variations in the physical and functional properties of grains and also on the agro-climatic conditions such as type of soil, location, varietal differences, climate, availability of water etc. [13] and [14] found the similar values for all the proximate principles with slight variations.

3.2 Mineral composition of colored horse gram varieties

Table 2 represents the mineral composition of colored horse gram varieties. There was significant difference ($p < 0.01$) between the colored horse gram varieties for calcium, iron, copper, zinc and manganese content. The calcium, iron, copper and zinc content was significantly higher in black colored variety (263.33, 8.64, 1.27 and 2.78 mg /100 g) followed by brown colored variety (245.01, 8.17, 1.22 and 2.70 mg /100 g) and cream colored variety (224.66, 7.07, 1.12 and 2.54 mg/ 100 g). The manganese content was significantly higher in black- and brown-colored varieties (3.15 and 3.12 mg/ 100 g) followed by cream-colored varieties (3.06 mg/ 100g) respectively. When compared to the control all the minerals in black-colored variety were high whereas less in cream colored variety. _The adequate mineral composition helps to combat the micro nutrient deficiency in developing countries like India. Horse gram is known for its good source of calcium and iron. The reason for the variation is may be due to the mineral content of the soil in which they are grown, the type of pesticide applied, the ability of the root to absorb minerals from the soil, the type of water used to grow and total ash content of that particular variety.

3.3 Nutraceutical content of colored horse gram varieties

Nutraceutical content of colored horse gram varieties is shown in Table 3. There was significant difference ($p < 0.01$) between the colored horse gram varieties for polyphenol, tannin and antioxidant activity whereas there is no significant difference between the colored horse gram varieties for soluble, insoluble and total dietary fiber content. The polyphenol, tannin and antioxidant activity of black colored variety was significantly higher (329.60 mgGAE /100 g, 315.20 mg/ 100 g and 88.45 %) compared to brown (241.45 mgGAE /100 g, 226.03 mg/ 100 g and 81.28 %) and cream colored variety (146.22 mgGAE /100 g, 135.57 mg/ 100 g and 75.35 %) respectively. When compared to the control the polyphenol, tannin and antioxidant activity was high for black whereas less for cream-colored variety. Presently phenols and tannins are considered as important dietary antioxidants [15]. They act as reducing agents (free radical terminators), metal ion chelators and singlet oxygen quenchers [16] and thus prevent oxidative damage to biomolecules such as DNA, lipids and proteins. Seed coat is usually expected to contain a higher amount of polyphenolic compounds as expected from their protective function in plant foods. Polyphenols, tannins and antioxidant activity of black-colored horse gram were significantly higher ($p< 0.01$) followed by brown and cream-colored horse gram variety. This may be attributed to variations in the colour of the seed coat. It was also noticed that the color of the seed coat could also significantly influence the antioxidant activity (Table 3). There was a significant difference in the antioxidant activity where black-colored horse gram showed the highest activity

followed by brown and cream-colored horse gram varieties. The soluble, insoluble and total soluble fiber content in colored varieties ranged from 1 to 1.03 per cent, 27.70 to 27. 74 per cent and 28.70 to 28.77 per cent respectively. A sufficient amount of dietary fiber is required for the proper functioning of the gut and has also been associated with a reduction for a number of non-communicable diseases such as heart disease, certain cancers and diabetes. [17] and [13] reported total dietary fiber content of 26.8 and 22.14 g per 100g in horse grams respectively.

3.4 Antinutrient content of colored horse gram varieties

Table 4 illustrates the antinutrient content of colored horse gram varieties. A significant difference ($p < 0.01$) was observed between the colored horse gram varieties for phytic acid and oxalic acid content. The highest phytic acid and oxalic acid content was registered in black colored variety (12.22 and 4.66 mg/ g) followed by brown colored variety (11.44 and 4.46 mg/ g) and cream colored variety (10.94 and 4.66 mg/ g). When compared to control the phytic acid and oxalic acid content was high for black whereas less for cream-colored variety. The presence of antinutrients like phytic acid and oxalic acid in horse gram forms complexes with other minerals to form an insoluble salt and renders the non-bioavailability of certain important nutrients like calcium, zinc, iron, and magnesium. Apart from this, antinutrients also inhibit the digestibility of proteins and promotes 'hard-to-cook' properties in pulses [18]. _Differences in anti-nutrient content may be attributed to varietal differences, differences in physical properties, conditions of crop cultivation, or the amount of pesticide used on the crop.

4. CONCLUSION

Among the proximates, protein and crude fiber contents were high in brown followed by black and cream-colored varieties respectively. The calcium, iron, copper and zinc content were significantly higher in black-colored variety followed by brown-colored variety and cream colored variety. The polyphenol, tannin and antioxidant activity of black colored variety was high compared to the brown and cream colored variety. The high amount of antinutrients *i.e.,* phytic acid and oxalic acid was found in black colored variety followed by the brown and cream-colored variety. Thus, horse gram must be pre-processed prior to consumption to improve its nutritional quality along with increasing the bioavailability of nutrients

5. Future scope of the study

- Effect of different processing on nutritional, antinutritional and nutraceutical properties of colored horse gram
- Development and quality evaluation of horse gram-incorporated food products
- Effect of different processing on in-vitro starch and protein digestibility of colored horse gram

Conflict of interest

The authors of the present study confirms that there is no conflict of interest related to this work.

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Black colored horse gram *Cream colored horse gram* *Brown colored horse gram*

Figure 1: Different colored horse gram varieties

Table 1. Proximate composition of flour from colored horse gram varieties

Horse gram variety	Proximate principles (g %)							
	Moisture	Fat	Protein	Crude fiber	Ash	Total CHO#	Available CHO#	Energy (Kcal)
Brown (Control)	8.31±0.10 c	0.76±0.01 a	24.50± 0.11 a	6.11 ±0.13 a	3.24± 0.03 b	57.07±0.14 c	63.19±0.02 c	333 ±0.57 a
Cream	9.20±0.10 a	0.79±0.01 a	21.30± 0.10c	5.29 ±0.06 c	3.18± 0.02 c	60.22±0.14 a	65.51±0.09 a	333± 0.57 a
Black	8.70 ±0.10 b	0.68±0.02 b	22.50 ±0.11 b	5.85 ±0.06 b	3.33± 0.05 a	58.92±0.10 b	64.78±0.07 b	332± 0.57 b
C.D.	0.11**	0.03**	0.11**	0.10**	0.05**	0.15**	0.08**	0.66**
S. Em.±	0.03	0.01	0.03	0.02	0.01	0.04	0.02	0.19
F value	61.66	28.21	784	62.22	10.89	424.91	881.64	8.33

#CHO- carbohydrate

*Note: Values are mean ± S. D. of three replications, Values with same superscript in the same column are not significantly different from each other, S.Em: Standard Error of Mean, C. D: Critical Difference, **Significant @ 1%, NS-Non significant*

Table 2. Mineral composition of colored horse gram varieties

Horse gram variety	Mineral composition (mg %)				
	Calcium	Iron	Copper	Zinc	Manganese
Brown (Control)	245.01±2.71 ^{b}	8.17±0.01 ^{b}	1.22±0.02 ^{b}	2.70±0.02 ^{b}	3.12±0.01 ^{a}
Cream	224.66±2.51 ^{c}	7.07±0.02 ^{c}	1.12±0.02 ^{c}	2.54±0.02 ^{c}	3.06±0.02 ^{b}
Black	263.33±2.51 ^{a}	8.64±0.04 ^{a}	1.27±0.01 ^{a}	2.78±0.02 ^{a}	3.15± 0.02 ^{a}
C.D.	3.09**	0.03**	0.03**	0.03**	0.03**
S. Em.±	0.89	0.01	0.01	0.01	0.01
F value	155.4	260.72	54.80	112.28	18.61

*Note: Values are mean± S. D. of three replications, Values with same superscript in the same column are not significantly different from each other, S.Em: Standard Error of Mean, C. D: Critical Difference, **Significant @ 1%, NS-Non significant*

Table 3. Nutraceutical content of colored horse gram varieties

Horse gram variety	Antioxidant contents		Antioxidant activity (% inhibition)	Dietary fiber (g/ 100 g)		
	Polyphenols (mgGAE/100g)	Tannins (mg/100g)		Soluble fiber	Insoluble fiber	Total fiber
Brown (Control)	241.45 ± 0.22 ^{b}	226.03 ±0.85 ^{b}	81.28 ±0.05 ^{b}	1.02±0.01	27.72± 0.01	28.73± 0.01
Cream	146.22 ± 0.59 ^{c}	135.57 ±0.15 ^{c}	75.35 ±0.06 ^{c}	1.00±0.05	27.70± 0.01	28.70±0.01
Black	329.60 ± 0.25 ^{a}	315.20± 0.52 ^{a}	88.45± 0.10 ^{a}	1.03±0.01	27.74± 0.05	28.77± 0.01
C.D.	0.78**	1.16**	0.15**	NS	NS	NS
S. Em.±	0.22	0.33	0.04	0.01	0.01	0.01
F value	1631.81	7074.53	2175.36	104.08	1799.90	9888.41

*Note: Values are mean ± S.D. of three replications, Values with same superscript in the same column are not significantly different from each other, S.Em: Standard Error of Mean, C. D: Critical Difference, **Significant @ 1%, NS-Non significant*

Table 4. Antinutrients content of colored horse gram varieties

Horse gram variety	Phytic acid (mg/g)	Oxalic acid (mg/g)
Brown (Control)	11.44± 0.07 ^{b}	4.46±0.09 ^{b}
Cream	10.94± 0.08 ^{c}	4.23±0.09 ^{c}
Black	12.22± 0.10 ^{a}	4.66±0.11 ^{a}
C.D.	0.17*	0.19*
S. Em.±	0.05	0.05
F value	163.61	8.48

*Note: Values are mean ± S.D. of three replications, Values with same superscript in the same column are not significantly different from each other, S.Em: Standard Error of Mean, C. D: Critical Difference, *Significant @ 5%*

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