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Effect of Cooking on Proximate, Phytochemical, Anti-oxidant and Enzyme Inhibitory Activity of Horse Gram (*Macrotyloma uniflorum*)

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ABSTRACT: Horse gram (*Macrotyloma uniflorum*) is an underutilized and unexplored food legume. The horse gram is consumed mostly in the form of boiled and roasted. Even though, the horse gram is a richest source of phytochemical, the bioactive compounds are lost significantly during cooking process. The effects of cooking on proximate, anti-oxidant and enzyme inhibitory activity of horse gram were evaluated. The study was carried out to find the suitable method of cooking to be followed to reduce the nutrients loss and retain the maximum amount nutrients horse gram. During. The total phenol and flavonoid content of the cooked horse gram were in the range of 120-268.14 mg GAE/100 g and 331.82-1340.91 mg QE/100 g respectively. The lowest total phenol and flavonoid was recorded in pan boiled and pressure-cooked horse gram respectively. The DPPH and ABTS free radical scavenging activity was significantly increased during roasting of horse gram, whereas the anti-oxidant activity was significantly reduced during pan boiling and pressure-cooking method. The α-amylase inhibitory activity of cooked horse gram was in the range of 25.33-38.41 per cent whereas α-glucosidase inhibition activity was between the range of 35.67 to 47.20 per cent. The α-amylase and α-glucosidase inhibition activity were significantly decreased during cooking of horse gram. The raw horse gram had highest α-amylase and α-glucosidase inhibition activity when compared to cooked samples.

Keywords: Horse gram, Pressure cooking, Boiling, Roasting, cooking.

INTRODUCTION

Horse gram (*Macrotyloma uniflorum*) is a relatively unexplored leguminous crop that is primarily grown in tropical Africa and Southeast Asia. It possesses a wide variety of advantageous qualities and can be used in a variety of culinary applications. However, horse gram is native to the southern region of India, the name "*Macrotyloma*" comes from the Greek words "*makros*" for huge, "*tylos*" for knob, and "*loma*" for margin; it refers to their knobby statures on the pods. India, Malaysia, Myanmar, Nepal and Mauritius, and Sri Lanka are the primary producer of the crop. It is cultivated as a fodder crop in Australia and Africa. It is regarded as "poor man's meal", especially in South India, despite their significant contribution to the diet of the Indian population. In comparison to other pulses of higher importance, horse gram has received a lower level of agronomical research attention (Ingle *et al.*, 2020). In India, horse gram grown over an area of 0.348 million ha, producing 0.226 million tonnes with a productivity of 650 kg per ha in the years 2020–21. Horse gram production was 0.70 million tonnes during 1977–78 from an area of 2.002 million ha, which has decreased to 0.226 million tonnes and 0.348 million ha in the year 2020-21. It is mostly grown in the states of Karnataka, Tamil Nadu, Odisha, Andhra Pradesh, Chhattisgarh, Jharkhand, Uttarakhand and Bihar in India. Tamil Nadu is the second largest producer of horse gram after Karnataka. Tamil Nadu and Andhra Pradesh together account for nearly 90 per cent of the total production of horse gram in India (Indiastat, 2020-2021). The horse gram is consumed mostly in the form of boiled and roasted. Thermal processes like cooking structurally modifies the legume seeds but also modifies functional properties which in turn increases digestibility of seeds. Several reports showed significant decrease in certain heat-labile nutrients during cooking of seeds. Even though, the horse gram is a richest source of phytochemical, the bioactive compounds are lost significantly during cooking process. Several researches have been carried out on changes during boiling and pressure cooking of horse gram. But changing during steaming and microwave boiling of horse gram has not been reported. Hence, the study was carried to evaluate the effect of different domestic methods of cooking including steaming and microwave cooking on proximate composition, phytochemical content, anti-oxidant activity and enzyme inhibition activity of horse gram.

MATERIALS AND METHOD

Procurement of horse gram samples. Horse gram was procured from Regional Research Station, Tamil Nadu Agricultural University, Paiyur, Tamil Nadu.

Cooking of horse gram

Soaking of horse gram. The horse gram was washed thrice in water to remove extraneous material sticking to the seed coats. Then, the horse gram seeds were soaked in distilled water (1:4 w/v) for 12 h at room temperature. After soaking, the seeds were washed with water and subjected to different methods of cooking such as pan boiling, pressure cooking, roasting, steaming, microwave roasting and microwave boiling.

Pan boiling. The soaked horse gram seeds and distilled water were taken in conical flask in the ratio of 1:6 (w/v) and cooked on a hot plate until they became soft. The horse gram took 24 min to get cooked. After cooking, the grains were spread on filter paper to absorb the moisture present on surface of the grains.

Pressure cooking. Overnight soaked horse gram seeds and distilled water were taken in a ratio of 1:3 (w/v) in conical flask and cooked in pressure cooker until it become soft. The horse gram was cooked for 5 min and then water was discarded and cooked grains were spread on filter paper to absorb the moisture present on surface of the grains.

Microwave boiling. Overnight soaked horse gram grains were placed in conical flask with distilled water in the ratio of 1:6 (w/v) and cooked in microwave oven (MG607APR, LG electronics India Pvt. Ltd., India) at 800 W until it become soft. After cooking, the filter paper was spread under the grain to absorb excess water present on the surface of the grains.

Steaming. The soaked horse gram seeds were steamed at 100 °C for 19 min. The cooked grains were spread on filter paper to cool the grains and absorb the surface moisture of the grains.

Roasting. The horse gram seeds were roasted in aluminium pan (for 3 min) until it reached crunchy texture. After roasting, the grains were spread on stainless steel tray to cool the grains and stored in air tight container.

Microwave roasting. Horse gram samples were placed in glass tray and roasted in microwave oven (MG607APR, LG electronics India Pvt. Ltd., India) at 800 W until it reaches crunchy textutre. Then, the roasted seeds were cooled and stored in air tight container.

After cooking, all the cooked samples were freeze dried, ground in to fine flour and storied at -20°C untill used for further analysis.

Proximate composition of horse gram. Moisture content was determined by weight loss of 5 g sample after heating at 110^{o}C for 2 hours. The ash content was measured by weight loss of 5 g of moisture free sample for heating at 550^{o}C for 5 hours. The crude fat content in the samples was determined by ether extraction using glass soxhlet. The crude protein was determined by using Micro Kjeldhal method. Carbohydrate content of the grain was determined by using anthrone method. Minerals content was estimated byatomic absorption spectroscopy (AAS) (AOAC, 2002).

Total phenol, flavonoid, tannin and anthocyanin content. The total phenolic content of horse gram samples was estimated by folin-ciocalteu method as described by Bajalan *et al.* (2016). The absorbance was measured at 760 nm and the total phenol content of the samples was expressed as milligrams of Gallic acid equivalent per 100 g of sample (mg of GAE/100 g). Total flavonoids content of horse gram samples was determined as method described by El-Haci *et al.* (2013). The absorbance was measured at 510 nm and the results were expressed as milligram of quercetin (QAE) equivalent per 100 g of sample. Condensed tannins were determined by vanillin-HCl method as described by Chavana *et al*. (2001). The absorbance was measured at 500 nm and the results were expressed as milligram of catechin equivalents (mg CAE/100 g) per 100 g of sample. Total anthocyanin content of the samples was analysed by pH differential method described by Asem *et al.* (2015). It was expressed as milligrams of cyanidin 3-glucoside equivalent/100 g (Cy-3-glcE/100 g) samples.

Anti-oxidant activity. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured using the method of Saharan *et al.* (2017). ABTS anti-oxidant activity of seed extract was measured by radical cation decolourization assay involving ABTS* radical cation given by Nithiyanantham *et al.* (2012). FRAP (Ferric Reducing Antioxidant Power) assay was carried out as described by Marathe *et al*. (2011).

Statistical Analysis. The experiments were performed in triplicates and data presented in mean ± standard error. The results were analysed using analysis of variance (ANOVA) (SPSS, 2002). The Duncan multiple range test was used to separate the means and accepted at the level of $P \leq 0.05$.

RESULT AND DISCUSSION

Effect of cooking on proximate composition of horse gram. The proximate composition of raw and cooked horse gram is given in Table 1. Moisture content of the sample is important in determination of shelf life of the products. A high moisture content causes storage issues because it promotes fungal and insect attack. The analysis found that that moisture content of the raw horse gram was 8.33 per cent. The moisture content of the cooked samples was in the range of 8.49-9.98 per cent. The roasted horse gram had lowest moisture content and pressure-cooked horse gram contained highest moisture content. The protein content of the samples was between the range of 20.36-24.67 per cent. The highest amount of protein loss was occurred during the pan boil cooking method due to the leaching of protein in cooking water. Fat content of the samples varied between of 0.79 and 1.07 per cent with highest in raw (1.07%) and lowest for pan boiled horse gram. Fiber content of the samples was in the range of 5.66–6.08 per cent with highest level in raw horse gram. Ash and carbohydrate content of raw and cooked horse gram were in the range of 1.56–2.93 and 54.00–58.90 per cent respectively. Similar result was reported by Sidduraj *et al*. (2008) and the cooking losses in nutrient was higher in moist heat method of cooking when compared to dry heat methods. Vashishth *et al*. (2021) evaluated the effect of different cooking methods on the proximate composition of horse gram. The protein content of cooked GPM-6 and paiyur 2 horse gram were ranged from 22.05 to 24.67 % and 23.80 to 24.96 respectively. The fat content of cooked GPM-6 and paiyur 2 horse gram was in the range of 0.96-0.27 % and 1.09-1.24 % respectively. The ash and carbohydrate content of cooked GPM-6 horse gram were in the range of 2.61-2.84 % and 63.29-65.35 % respectively. The paiyur 2 horse gram had ash and carbohydrate in the range of 2.61-2.78 % and 63.03-6.04 % respectively. The study reported that protein, ash and carbohydrate content of horse gram grain were significantly ($p \leq 0.05$) decreased during processing. The similar results were observed in the present study.

Table 1: Proximate composition of raw and cooked horse gram.

Samples	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)
Raw	8.33±0.19[a]	24.67±0.58[e]	1.07±0.02[e]	6.08±0.11[a]	2.93±0.02[f]	58.90±0.32[c]
Pan boiled	9.65±0.15[d]	20.36±0.21[a]	0.79±0.04[a]	5.66±0.05[b]	1.56±0.02[a]	54.00±1.03[a]
Pressure cooked	9.98±0.21[d]	21.63±0.39[b]	0.84±0.01[b]	5.69±0.18[b]	1.89±0.03[b]	56.66±0.58[bc]
Steaming	8.49±0.16[a]	23.67±0.51[cd]	0.98±0.02[d]	5.79±0.02[b]	2.63±0.07[d]	58.01±1.33[c]
Roasted	9.06±0.13[b]	22.88±0.73[c]	0.97±0.01[d]	5.71±0.09[b]	1.98±0.05c	57.23±0.27[bc]
Microwave boiled	9.73±0.10[d]	21.01±0.75[ab]	0.87±0.01[bc]	5.78±0.08[b]	1.60±0.03[a]	55.01±0.38[ab]
Microwave roasted	8.51±0.07[a]	23.98±0.86[de]	0.91±0.02[c]	5.81±0.09[b]	2.74±0.07[e]	57.99±0.28[c]

Effect of cooking on phytochemical content of horse gram. The total phenol, flavonoids, tannins and anthocyanin content of the raw and cooked horse gram were estimated and presented in Table 2. The total phenol, flavonoids, tannins and anthocyanin content of raw horse gram was 252.20 mg GAE/100 g, 1240.91 mg QE/100 g, 1546.34 mg CE100 g and 3.67mg Cy-3-glcE/100 g. The total phenol and flavonoid content of the cooked horse gram were in the range of 120-268.14 mg GAE/100 g and 331.82-1340.91 mg QE/100 g respectively. The lowest total phenol and flavonoid was recorded in pan boiled and pressure-cooked horse gram respectively. The tannin and anthocyanin content were between the range of 226.45 to 1466.2334 mg CE/100 g and 0.57 to 3.67 mg Cy-3-glcE/100 g respectively. The losses of tannin and anthocyanin content was highest in pressure cooked and steamed horse gram respectively. The hydrothermally treated seed sample had the lowest concentration of phenolic fractions, possibly due to the dissolution of phenolics in the soaking medium and subsequent discarding of them. Herath *et al.*, (2020) reported total phenol and total flavonoid content of black and brown horse gram. The total phenol and flavonoid content of brown gram were 1.30±0.03 and 0.87±0.07 mg/g while, black horse gram had 1.68±0.12 mg/g of total phenol and 1.35±0.13 mg/g of flavonoid. Ojha *et al*. (2020) reported that the tannin content of the whole horse gram was 11.75 mg/g. The tannin content roasted horse gram was decreased by 28.0 %. The study conducted by Vashishth *et al*. (2021) also reported that losses in tannins and flavonoids during cooking of horse gram. The total phenol and flavonoids content were significantly ($p \leq 0.05$) affected during processing. Extrusion processing and boiling and pressure cooking had shown the maximum reduction in phenolic content of horse gram. The tannins content in raw seeds of both PAIYUR-2 and GPM-6 varieties were 0.901% and 0.924 and respectively. The tannin content of the studied varieties was found to be lower than the reported value of black variety (1.58 /100 g) of horse gram. The tannin content was reduced by 58.49 – 65.59% and 51.19 – 67.53% during processing for PAIYUR-2 and GPM-6 varieties respectively. The change in extractable phenolic content during processing may be attributed to the change in solubility and degradation of phenols during thermal processing (Sreeramulu *et al.*, 2009).

Table 2: Phytochemical content of horse gram.

Sample	Total phenols (mg GAE/100 g)	Total flavonoids (mg QE/100 g)	Tannin (mg CE/100 g)	Anthocyanin (mg Cy-3-glcE/100 g)
Raw	252.20±1.48[c]	1240.91±10.23[f]	1546.34±39.17[f]	3.67±0.01[g]
Pan boiling	120.34±1.88[a]	477.27±8.44[c]	1166.98±7.93[d]	1.03±0.02[c]
Pressure cooking	122.37±3.33[a]	331.82±1.80[a]	226.45±1.69[a]	1.70±0.01[d]
Roasting	253.22±6.72[c]	1195.46±17.89[e]	1466.23±4.99[e]	2.91±0.07[f]
Steaming	124.07±1.43[a]	522.73±9.24[d]	346.90±9.89[b]	0.67±0.02[b]
Microwave boiling	268.14±5.10[d]	1340.91±36.49[g]	346.77±11.06[b]	2.47±0.01[e]
Microwave roasting	157.29±0.86[b]	431.82±0.59[b]	806.54±10.41[c]	0.57±0.003[a]

Effect of cooking on anti-oxidant activity of horse gram. The free radical scavenging activity of raw and cooked horse gram was determined by DPPH, ABTS and FRAB method and results are shown in Table 3. The antioxidant activity namely DPPH, ABTS and FRAB free radical scavenging activity of raw horse gram were 35.71 per cent, 54.31 per cent and 1566.08 mg dm/mmol Fe (II) respectively. The DPPH and ABTS free radical scavenging activity was significantly increased during roasting of horse gram, whereas the anti-oxidant activity was significantly reduced during pan boiling and pressure-cooking method. The DPPH and ABTS free radical scavenging activity of cooked horse gram were between the range of 7.52 to 44.72 per cent and 33.01 to 56.58 per cent respectively. The FRAB free radical scavenging activity of cooked horse gram was in the range of 576.61-1878.36 with highest in microwave cooked sample and lowest for pressure cooked horse gram. Similar study was conducted by Ojha *et al*. (2020). The study reported that the antioxidant activity of horse gram was 52.68 DPPH % free radical inhibition and it increased during roasting by 29.13%. Mathaiyan *et al*. (2021) reported the anti-oxidant potential of horse gram samples. Horse gram reduced the ferric and FRAP complex up to 98% at the concentration of 150 mg/ml. Similarly, the DPPH and phosphomolybdenum complex radicals were effectively scavenged by 93.14% and 95.42% at the concentration of 150 mg/ml respectively. The development of products from the Maillard reaction may contribute to the stability of antioxidant potency in dry heated samples. The roasted seeds had the highest antioxidant properties due to the development of products as a result of Maillard reaction (Nicoli *et al*., 1997). Due to phenolics diffusing into the cooking water during the cooking process from the seed coat, the low level of phenolic contents of pressure-cooked samples correlates with their decreased reducing power (Rocha-Guzman *et al.*, 2007). The further reduction in the antioxidant activity of the grains can be attributed to the combined effect of heat sensitivity and water solubility of phenols and other components which exhibit antioxidant potential.

Table 3: Anti-oxidant activity of raw and cooked horse gram.

Sample	DPPH (%)	ABTS (%)	FRAB mg dm/mmol Fe (II)
Raw	35.71±0.31[d]	54.31±1.05[d]	1566.08±44.29[d]
Pan boiling	11.77±0.27[b]	35.61±0.82[b]	678.95±11.54[b]
Pressure cooking	8.82±0.13[a]	33.01±0.31[a]	576.61±15.69[a]
Roasting	44.72±1.39[e]	56.58±0.23[e]	1263.16±5.15[c]
Steaming	11.97±0.35[b]	39.35±0.10[c]	681.87±15.77[b]
Microwave boiling	31.07±0.73[c]	55.93±0.53[e]	1878.36±20.44[e]
Microwave roasting	7.52±0.11[a]	40.49±0.13[c]	620.47±18.99[a]

Effect of cooking on enzyme inhibitory activity of horse gram. α-Amylase and α-glucosidase inhibitory action is essential for controlling hyperglycemia. (Vadivel and Biesalski 2011). The α-Amylase and α-Glucosidase Inhibition activity of raw horse gram was 55.16 and 65.50 per cent respectively (Table 4). The α-Amylase inhibitory activity of cooked horse gram was in the range of 25.33-38.41 per cent whereas α-Glucosidase Inhibition activity was between the range of 35.67 to 47.20 per cent. The α-Amylase and α-Glucosidase Inhibition activity were significantly decreased during cooking of horse gram. The raw horse gram had highest α-amylase and α-glucosidase inhibition activity when compared to cooked samples. The absorption of glucose in the intestine can be retarded by the use of α-glucosidase inhibitors. Similar to α-amylase inhibition activity and α-glucosidase inhibition activity also decreased during processing and found directly proportional to the phenolic content of the grains (Vashishth *et al*., 2022). The study reported that horse gram had considerable α-amylase (49.34 & 45.89%) and α-glucosidase (62.72 and 60.33%) inhibition activity. The inhibition activity of the grains reduced after processing and ranged from 12.40-30.63% and 14.13-33.37% for GPM-6 and Paiyur-2 respectively. Among cooked grains, the minimum reduction in α-amylase inhibition activity was observed for microwave cooked samples (33.25 and 32.36 %) for

GPM-6 and Paiyur-2 respectively. Among the cooked samples lowest reduction in α- glucosidase inhibition activity was found for microwave treated grains (30.83 and 25.66 %) for GPM-6 and paiyur-2 and respectively. The higher reduction of α-amylase inhibition potential in roasting and microwave roasting can be attributed to the change in activity of inhibitors as both the processing techniques imply the dry heating at high temperature during processing in comparison with other used cooking method (Sreerama *et al*., 2012).

Table 4: Enzyme inhibitory activity of raw and cooked horse gram.

Sample	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)
Raw	55.16±0.29[d]	65.50±0.30[e]
Pan boiling	34.57±1.05[b]	45.23±0.27[cd]
Pressure cooking	33.19±0.04[b]	43.61±1.00[c]
Roasting	25.33±0.68[a]	37.01±0.62[a]
Steaming	38.41±1.28[c]	40.01±0.63[b]
Microwave boiling	37.95±0.49[c]	47.20±0.13[d]
Microwave roasting	29.09±0.33[b]	35.67±0.12[a]

CONCLUSION

The present study reveals that the horse gram seeds possess appreciable levels of total phenols, flavonoids and tannin content. The horse gam also had functional properties such as antioxidant and enzymatic inhibition properties. The roasting and pressure cooking significantly (p≤0.05) affected the antioxidant and enzyme inhibitory activity of horse gram. Even though the processing of grain has shown the deleterious effects on the phenolic content and functional properties of grain but they still possess significant antioxidant potential and other functional properties. Hence, they can still be appreciably utilized as an important ingredient in daily diet. All the grains have shown a significant correlation between antioxidant potential and phenolic content of the grain.

FUTURE SCOPE

The present study helps in understanding the changing in proximate composition and other phytochemicals horse gram during domestic cooking of horse gram. The modern cooking methods such as extrusion, radiation and Micronization cooking (IRC) may be explored and compared with the conventional methods of cooking.

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Conflicts of Interest: None.

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