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TRADITIONAL PROCESSES INFLUENCING NUTRITIONAL AND ANTINUTRITIONAL FACTORS OF HORSE GRAM (MACROTYLOMA UNIFLORUM)

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Horse gram (*Macrotyloma uniflorum*), a traditional tropical legume is the cheapest source of protein and an excellent supply of minerals such as calcium, iron and molybdenum. However, its usage is limited due to its hardness, poor cooking quality and presence of an array of antinutritional factors (ANFs) such as phytates, trypsin inhibitor, hemagglutinins and polyphenols. Since many of these ANFs are toxic and indigestible, their reduction or elimination is inevitable before consumption of these plant foods. Hence, an attempt was made to analyse the proximate composition and antinutritional factors in horse gram by subjecting it to various traditional processes. The results showed an overall improvement in the energy level of pressure cooked legume. The minerals such as Ca, Mg, Cu, Mn, Fe, Zn, K, Mo and Na also increased when processed. There was a significant reduction in the antinutrients such as phytic acid (79%), total phenolics (76%), tannins (51%), flavonoids (83%) and trypsin inhibitors (64%). Total carotenoids and β-carotene content increased significantly when compared to control. The radical scavenging activity was increased in all the processed samples while there was a reduction in the reducing activity of all the samples. Hence, it is found from the present investigation that the traditional processes play a significant role in improving the nutritional value of horse gram.

Keywords: *Macrotyloma uniflorum*, Antinutritional factors, Nutritional value, Processes, Formulation, Malnourishment

INTRODUCTION

Legumes have a very specific place from the nutritive point of view and play an important role in nourishing world's population (Bojnanska *et al.*, 2012). In general, legumes are a source of complex carbohydrates, proteins and dietary fibres, having significant amounts of vitamins and minerals and hence are important in Indian diet (Tharanathan and Mahadevamma, 2003). Food legume consumption is found to be inversely associated with the risk of coronary heart diseases (Bazzano *et al*., 2001), type II diabetes mellitus (Villegas *et al.*, 2008), obesity (Rizkalla *et al*., 2002) and results in lower LDL cholesterol and higher HDL cholesterol (Bazzano *et al*., 2001; and Anderson and Major, 2002).

Horse gram (*Macrotyloma uniflorum*) is one such legume which is not commonly used for human consumption due to its poor cooking quality, although it is a good source of proteins and minerals (Sudha *et al*., 1995). However, it is a minor legume found to be nutritionally superior (Bravo *et al.*, 1999) which is used as a pulse crop and consumed as sprouts in many parts of India (Kadam *et al*., 1985).

It has recently been shown to prevent atherosclerosis in rats and also a potential functional food for the

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prevention of hyperlipidemic atherosclerosis (Shobana *et al*., 2012). Extracts from horse gram plants have shown potential for treating several human infections (Kawsar *et al*., 2008). The legume is extensively cultivated in dry areas of Australia, Burma, India, and Sri Lanka (Duke and Reed, 1981). Recently, this legume was identified in USA as a potential food source for the future (National Academy of Sciences, 1978). The legume is relatively high in iron, but its availability is reduced by the presence of phytates, trypsin inhibitors, haemagglutinins and polyphenols in it (Bhokre *et al*., 2012).

Many researchers have attempted to improve the cooking and nutritive properties through dehulling or milling and other processes. Phenolic compounds and other phytochemicals are abundant in seed coats of legumes that possess antinutritional properties. These antinutritional factors may hinder efficient utilization, absorption or digestion of nutrients and thus reduce their nutrient bioavailability and nutritional quality (Liener, 1975). Anemia and other mineral deficiency disorders are common in regions where primarily vegetarian diet is consumed (Erdman, 1979). The present investigation is aimed at comparing different traditional processes on the nutritional and antinutritional factors of horse gram.

MATERIALS AND METHODS

Horse gram (*Macrotyloma uniflorum*) procured from local market, was cleaned to remove broken grains and other extraneous materials. All standard chemicals were obtained from Sigma Chemicals (USA). Other chemicals, reagents, solvents used in this study were of analytical, extra pure and HPLC grade.

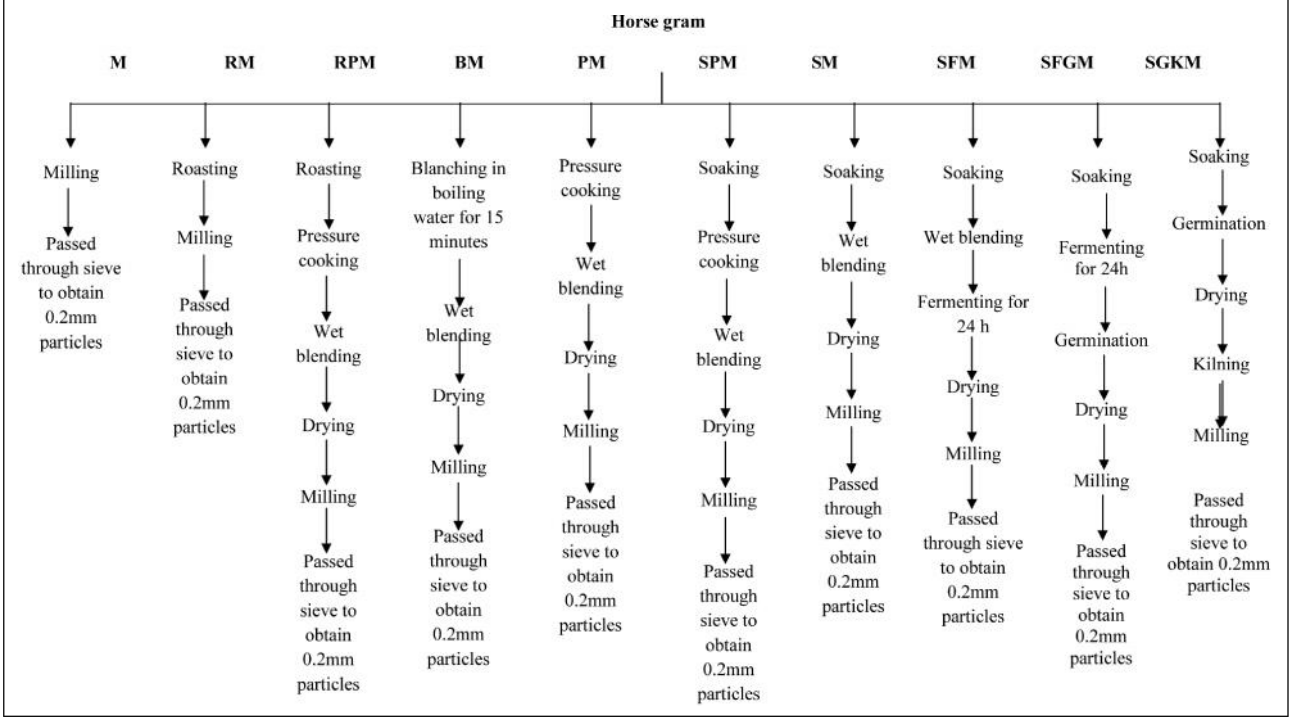
Sample Preparation

An aliquot of 25 g each of horse gram was subjected to nine different processes such as M-milling; RM-roasting & milling; RPM-roasting, pressure cooking & milling; BM-blanching & milling; PM-pressure cooking & milling; SPM-soaking, pressure cooking & milling; SM-soaking & milling; SFM-soaking, fermenting & milling; SFGM-soaking, fermenting, germinating & milling, SGKM-soaking, germinating, kilning & milling (Figure 1). All the samples were prepared accordingly and stored in cold for further analysis.

Proximate Analysis of Raw and Processed Horse Gram

Total sugar estimation was carried out by phenol-sulphuric acid method (Dubois *et al*., 1956) by preparing a standard graph for D-glucose with a working range of 0-25 µg/ml.

Figure 1: Processes Adopted in the Present Study



Total protein (N x 6.25), total lipid, minerals, moisture (AOAC, 2007) and crude fibre (Maynard, 1970) estimations were carried out.

Estimation of antinutrients

The phytic acid contents of all the processed samples were estimated by the method of Gao *et al.* (2007) using sodium phytate (Sigma chemicals, USA) as a standard. Total polyphenol extraction was carried out by the method of Chethan and Malleshi (2007) and estimated spectrophotometrically by the method of Singleton *et al.* (1995) using gallic acid (Sigma chemicals, USA) as a standard. The results were expressed as milligram gallic acid equivalents (mg GAE). Tannins were estimated by the method of Price *et al.* (1978) and flavonoids by Zhishen *et al.* (1999) using catechin (Sigma chemicals, USA) as a standard. The results were expressed as milligram catechin equivalents (mg CAE). Trypsin inhibitors were estimated by the method of Hamerstrand *et al*. (1981) using trypsin (Sigma chemicals, USA) as a substrate.

Antioxidant Properties

Antioxidant activities of polyphenol extract was tested through DPPH free radical scavenging activity and reducing power assay by the methods of Oyaizu (1986) and De Ancos *et al*. (2002) respectively.

HPLC Analysis of Carotenoids in the Extracted Oil

Carotenoids were separated on a C-18 Supelco HS20419BQ column (25 x 4.6 mm internal diameter). Mobile phase for separation of carotenoids contained acetonitrile: methanol: dichloromethane (60:20:20, v/v/v) and 0.1% ammonium acetate. Injected samples (20 µl) were maintained under isocratic condition at a flow rate of 1ml/min. β-carotene and lutein obtained from Sigma Chemicals (USA) were used as standards.

Statistical Analysis

Data, expressed as mean ±SD was statistically analyzed using one-way ANOVA. Duncan's multiple tests were used to compare means and significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The processed samples of horse gram (as represented in flow chart) were subjected to proximate analysis using standard procedures. Total carbohydrates in raw and processed horse gram significantly varied ranging from 44.2 to 86.2% with 64.3% in raw sample and significantly highest

Table 1: Nutritional Composition of Raw and Processed Horse Gram (*Macrotyloma uniflorum*)

Process	Total Sugars	Total Protein	Total Fat	Crude Fiber	Ash	Moisture	Energy (K cal)
			Dry Weight Basis (%)				
M (control)	64.3 ± 0.012^{c}	24.5 ± 0.009^{c}	0.8 ± 0.007^{c}	4.69 ± 0.002^{c}	2.8 ± 0.006^{c}	7 ± 0.007^{c}	331.78^{a}
RM	62.8 ± 0.011^{c}	27.15 ± 0.02^{c}	0.2 ± 0.008^{a}	3.56 ± 0.014^{a}	3.2 ± 0.009^{c}	4 ± 0.011^{a}	368.72^{b}
RPM	74.9 ± 0.009^{b}	29.77 ± 0.014^{b}	0.2 ± 0.014^{a}	4.17 ± 0.013^{c}	2.6 ± 0.01^{c}	6 ± 0.012^{c}	428.82^{b}
BM	74.8 ± 0.01^{b}	20.14 ± 0.013^{c}	0.5 ± 0.013^{c}	3.56 ± 0.007^{a}	2.8 ± 0.023^{c}	4 ± 0.013^{a}	391.38^{b}
PM	85.3 ± 0.013^{b}	32.45 ± 0.012^{b}	0.4 ± 0.022^{a}	3.03 ± 0.012^{a}	5.2 ± 0.018^{b}	3 ± 0.013^{a}	480.66^{b}
SPM	86.2 ± 0.008^{b}	24.5 ± 0.011^{c}	0.5 ± 0.013^{c}	4.08 ± 0.013^{c}	2.4 ± 0.014^{c}	4 ± 0.012^{a}	445.46^{b}
SM	64.9 ± 0.011^{c}	25.4 ± 0.012^{c}	0.4 ± 0.018^{a}	3.03 ± 0.009^{a}	3.4 ± 0.011^{b}	3 ± 0.011^{a}	370.86^{b}
SFM	44.2 ± 0.012^{a}	28.03 ± 0.016^{c}	0.5 ± 0.017^{c}	5.19 ± 0.017^{b}	2.8 ± 0.014^{c}	8 ± 0.009^{c}	379.48^{b}
SFGM	75.2 ± 0.02^{b}	24.5 ± 0.015^{c}	0.6 ± 0.011^{c}	4.08 ± 0.012^{c}	5.0 ± 0.012^{b}	4 ± 0.012^{a}	412.36^{b}
SGKM	84.8 ± 0.012^{b}	29.78 ± 0.012^{b}	0.5 ± 0.012^{c}	3.53 ± 0.014^{a}	3.8 ± 0.011^{b}	2 ± 0.013^{a}	469.88^{b}

Note: Abbreviations: M - Milling; RM - Roasting & Milling; RPM - Roasting, Pressure cooking & Milling; BM - Blanching & Milling; PM - Pressure cooking & Milling; SPM - Soaking, Pressure cooking & Milling; SM - Soaking & Milling; SFM - Soaking, Fermenting & Milling; SFGM - Soaking, Fermenting, Germinating & Milling, SGKM - Soaking, Germinating, Kilning & Milling. All values are mean of triplicates with SEM and are significant if the column not sharing same alphabets in the table.

in SPM followed by PM (86.2%) (Table 1). This is in agreement with earlier findings (Sreerama *et al*., 2012). The lowest in SFM (44.2%) may be due to utilization of the available carbohydrates as a source of carbon by fermenting microorganisms. During germination, mobilization and hydrolysis of the seed polysaccharide occur that can be further hydrolysed by fermenting microbes possessing both α & β amylases (Khetarpaul and Chauhan 1990). Heat treatment is shown to increase susceptibility of starch to amylase hydrolysis by promoting hydration, loss of structural integrity and partial solubilization of starch molecules (Garcia-Alonso *et al*., 1999; and Urooj and Puttaraj, 1999).

The total protein content in horse gram ranged from 20.14 to 32.45% with highest being in PM sample (Table 1). Raw sample had 24.5% protein with a similar range reported by Sreerama *et al*. (2012). Highest protein content found in PM samples indicates the free availability and retention of complex protein. The lowest protein content was in BM which may be due to rapid exposure to high temperature of blanching.

Horse gram had the fat content of 0.8% in raw sample which still reduced when subjected to different processes as obtained in the present study. However, significant reduction in the fat content was observed in RM and RPM samples indicating liquification and release of fat at the time of roasting, hence it is not accountable. Sudha *et al.* (1995) have reported fat content ranging from 0.7-2% in different varieties of horse gram. Legumes generally contain low fat ranging from 1-2% with the exception of chickpea-6.7% (Almeida-Costa *et al.,* 2006), soybean- 21% and peanut-4.9% (Augustine and Klein, 1989). Though, variation in fat content is due to varietal difference, lower lipid content of horse gram makes it suitable as one of the ingredients in weight restricting diets and also in improving the shelf life and keeping qualities of the grain (Sreerama *et al.*, 2012).

Crude fibre content of legumes ranged from 1.6-7.9% (Adebowale and Lawale, 2003). The value falls within this range in the present findings also. There was a slight increase in SFM sample (5.19%) when compared to control sample (4.69%). Reduction in crude fiber in RPM may be due to rapid exposure of the grains to high temperature.

In the present investigation, ash content ranged from 2.4-5.2% with highest in PM sample. In the earlier reports it ranged from 3.4-4% for beans (Augustine and Klein, 1989), but lower than 9.8% for chickpea & 10.4% for pea (Almeida-Costa *et al*., 2006). Kakati *et al*. (2010) have shown a significant variation in the moisture content of processed green gram and black gram, where the highest moisture content was in pressure cooking followed by soaking, germination and control. Similarly in the present investigation, moisture content was high in SPM (8%) which was highest when compared to raw sample of horse gram. Although there was variation in the nutrient content of variously processed grains, the energy level significantly increased when compared to their respective raw samples.

The ash obtained from all the processed grains of horse gram were subjected to AAS and the contents of Ca, Mg, Cu, Mn, Fe, Zn, K and Na were estimated. It was found that except Fe all other minerals were increased when compared to its raw sample (Table 2). Khetarpaul and Chauhan (1990) have also shown that fermentation at 48 h was more effective in increasing the extractability of trace elements like Cu, Zn and Mn and further increased on germination.

The extracted oils/fats were subjected to HPLC for the analysis of carotenoids. Total carotenoid was significantly high in case of RM (63.17 µg/g) when compared to raw samples (34.58 µg/g). β-carotene content was increased significantly in RM (6.59 µg/g) when compared to control sample (4.02 µg/g). This may be due to its significantly highest fat content, but lutein was reduced in all the processed samples (Table 3). Reduction in the total carotenoid content in some of the processed horse gram may be attributed to light exposure and also due to their instability to high temperature during fat extraction and sample preparation for HPLC. Khattak *et al.* (2008) have shown significant effect of germination and exposure to different kinds of light on β-carotene, protein solubility and protein digestibility of legumes.

All the processed samples of horse gram were also used for the estimation of antinutritional factors such as phytic acid, total polyphenols, tannins, flavonoids and trypsin inhibitors. The results are summarized in Table 4. Phytic acid content was significantly reduced in all the processed samples except SM in which it was increased when compared to its raw sample. This increase might be due to the usage of soaking water for grinding. Highly significant reduction was observed in SGKM (29.26 mg/100 g) when compared to control sample (231.76 mg/100 g). This reduction may be attributed to increased activity of endogenous phytase in the legumes during germination. Shimelis and Rakshit (2007) have reported over 75% reduction in the phytic acid content of three varieties of kidney beans.

Table 2: Mineral Contents (mg/100 g) of Raw and Processed Horse Gram (*Macrotyloma uniflorum*)

Process	Calcium	Magnesium	Copper	Manganese	Iron	Zinc	Potassium	Sodium
M (control)	20.40 ± 0.011^{c}	23.05 ± 0.01^{c}	1.03 ± 0.015^{c}	1.77 ± 0.008^{c}	7.38 ± 0.001^{d}	3.41 ± 0.017^{c}	10.60 ± 0.006^{c}	0.80 ± 0.011^{c}
RM	20.51 ± 0.009^{c}	24.81 ± 0.006^{c}	1.28 ± 0.002^{b}	1.85 ± 0.006^{c}	6.12 ± 0.013^{c}	4.09 ± 0.015^{b}	10.87 ± 0.007^{c}	0.63 ± 0.014^{a}
RPM	28.92 ± 0.013^{b}	28.77 ± 0.007^{b}	1.22 ± 0.006^{b}	2.32 ± 0.007^{b}	5.70 ± 0.014^{a}	4.03 ± 0.016^{b}	7.19 ± 0.011^{a}	1.00 ± 0.014^{c}
BM	19.08 ± 0.011^{c}	21.07 ± 0.009^{c}	3.50 ± 0.007^{a}	1.35 ± 0.004^{c}	5.00 ± 0.009^{a}	2.65 ± 0.008^{a}	8.14 ± 0.009^{a}	1.21 ± 0.009^{b}
PM	22.26 ± 0.012^{c}	19.81 ± 0.011^{c}	0.95 ± 0.011^{c}	1.71 ± 0.011^{c}	6.00 ± 0.007^{a}	3.31 ± 0.011^{c}	10.00 ± 0.008^{c}	1.06 ± 0.012^{b}
SPM	24.56 ± 0.01^{b}	25.57 ± 0.008^{c}	0.96 ± 0.009^{c}	1.61 ± 0.012^{c}	4.45 ± 0.012^{a}	2.83 ± 0.012^{a}	8.07 ± 0.007^{a}	2.70 ± 0.015^{b}
SM	24.26 ± 0.008^{b}	26.03 ± 0.011^{c}	1.73 ± 0.011^{b}	1.73 ± 0.007^{c}	6.96 ± 0.011^{c}	3.10 ± 0.009^{c}	10.28 ± 0.001^{c}	0.53 ± 0.014^{a}
SFM	23.03 ± 0.011^{b}	26.80 ± 0.008^{c}	1.78 ± 0.012^{b}	1.60 ± 0.005^{c}	6.17 ± 0.010^{c}	2.63 ± 0.006^{a}	8.90 ± 0.021^{a}	0.80 ± 0.008^{c}
SFGM	22.01 ± 0.011^{c}	25.70 ± 0.011^{c}	0.63 ± 0.013^{c}	1.85 ± 0.006^{c}	5.86 ± 0.009^{a}	3.02 ± 0.007^{c}	9.50 ± 0.009^{a}	0.62 ± 0.006^{a}
SGKM	24.75 ± 0.014^{b}	28.43 ± 0.013^{b}	1.34 ± 0.010^{c}	1.80 ± 0.011^{c}	6.17 ± 0.005^{c}	3.50 ± 0.008^{c}	11.42 ± 0.017^{b}	0.64 ± 0.011^{a}

Note: Abbreviations: M - Milling; RM - Roasting & Milling; RPM - Roasting, Pressure cooking & Milling; BM - Blanching & Milling; PM - Pressure cooking & Milling; SPM - Soaking, Pressure cooking & Milling; SM - Soaking & Milling; SFM - Soaking, Fermenting & Milling; SFGM - Soaking, Fermenting, Germinating & Milling, SGKM - Soaking, Germinating, Kilning & Milling. All values are mean of triplicates with SEM and are significant if the column not sharing same alphabets in the table.

Table 3: Carotenoid Content (µg/g) of Raw and Processed Horse Gram

Process	Lutein	β-carotene	Total Carotenoids
M (control)	3.500 ± 0.005^{b}	4.02 ± 0.003^{a}	34.58 ± 0.008^{c}
RM	_	6.59 ± 0.005^{b}	63.17 ± 0.006^{b}
RPM	0.050 ± 0.004^{a}	_	09.59 ± 0.005^{a}
BM	0.012 ± 0.006^{a}	_	11.33 ± 0.004^{a}
PM	0.027 ± 0.002^{a}	_	19.89 ± 0.003^{a}
SPM	0.243 ± 0.003^{a}	0.55 ± 0.005^{b}	23.96 ± 0.004^{a}
SM	0.117 ± 0.004^{a}	_	21.74 ± 0.003^{a}
SFM	0.660 ± 0.005^{a}	_	34.23 ± 0.002^{c}
SFGM	_	_	_
SGKM	0.740 ± 0.007^{a}	_	09.1 ± 0.002^{a}

Note: Abbreviations: M - Milling; RM - Roasting & Milling; RPM - Roasting, Pressure cooking & Milling; BM - Blanching & Milling; PM - Pressure cooking & Milling; SPM - Soaking, Pressure cooking & Milling; SM - Soaking & Milling; SFM - Soaking, Fermenting & Milling; SFGM - Soaking, Fermenting, Germinating & Milling, SGKM - Soaking, Germinating, Kilning & Milling. All values are mean of triplicates with SEM and are significant if the column not sharing same alphabets in the table.

Total polyphenols in horse gram was significantly reduced in all the samples and highest reduction was in SPM sample (127.1 mg GAE/100 g), while control sample had 544.5 mg GAE/ 100 g. Tannins and flavonoids content of raw and processed horse gram were estimated and expressed as catechin equivalents. Tannin content increased in RPM, PM, SM, SGKM and RM when compared to control sample while BM, SPM, SFM and SFGM showed a reduction. In the earlier findings, this reduction in tannin content was attributed to the formation of hydrophobic complex of tannins with seed proteins and enzymes (Sharma and Sehgal, 1992). This is also in agreement with the present investigation. Some loss may also be due to leaching of tannins into blanching and soaking water.

Flavonoid content decreased in all the samples and highly significant reduction was in SM (1.62 mg CAE/100 g) compared to control (9.26 mg CAE/100g). Similarly, trypsin inhibitor content also reduced significantly in all the samples while highest reduction was in RPM (76.3 mg/g) when compared to control (213.2 mg/g). This is in agreement with the previous report of Shimelis & Rakshit (2007) on kidney bean. The overall results revealed reduction in all the tested antinutrients of horse gram when subjected to various processes.

Antioxidant activity such as DPPH radical scavenging and reducing activity of all the horse gram samples were

Table 4: Antinutritional Factors of Raw and Processed Horse Gram

Process	Phytic Acid (mg/100 g)	Total Polyphenols (mg GAE/ 100 g)	Tannins (mg CAE/ 100 g)	Flavonoids (mg CAE/ 100 g)	Trypsin Inhibitors (mg/g)
M (control)	231.76 ± 0.007^{d}	544.5 ± 0.006^{b}	5.9 ± 0.009^{c}	9.26 ± 0.001^{b}	213.2 ± 0.002^{b}
RM	47.75 ± 0.003^{a}	214.6 ± 0.006^{a}	6.6 ± 0.007^{c}	3.23 ± 0.003^{a}	92.1 ± 0.004^{a}
RPM	124.84 ± 0.003^{a}	143.4 ± 0.005^{a}	9.4 ± 0.003^{b}	6.03 ± 0.004^{a}	76.3 ± 0.005^{a}
BM	185.45 ± 0.005^{c}	241.6 ± 0.002^{a}	4.8 ± 0.007^{c}	2.35 ± 0.007^{a}	100 ± 0.004^{a}
PM	165.84 ± 0.004^{c}	311.3 ± 0.004^{a}	8.5 ± 0.004^{b}	5.59 ± 0.006^{a}	128.9 ± 0.003^{a}
SPM	185.45 ± 0.007^{c}	127.1 ± 0.003^{a}	3.9 ± 0.005^{a}	4.85 ± 0.006^{a}	126.3 ± 0.006^{a}
SM	361.41 ± 0.006^{b}	369.1 ± 0.004^{c}	7.9 ± 0.008^{b}	1.62 ± 0.005^{a}	89.5 ± 0.003^{a}
SFM	48.76 ± 0.003^{a}	239.4 ± 0.007^{a}	2.9 ± 0.004^{a}	2.65 ± 0.007^{a}	97.4 ± 0.004^{a}
SFGM	47.75 ± 0.002^{a}	223.9 ± 0.009^{a}	3.7 ± 0.006^{a}	4.41 ± 0.008^{a}	155.3 ± 0.005^{c}
SGKM	29.26 ± 0.005^{a}	246.9 ± 0.005^{a}	7.2 ± 0.005^{c}	1.76 ± 0.004^{a}	94.7 ± 0.003^{a}

Note: Abbreviations: M - Milling; RM - Roasting & Milling; RPM - Roasting, Pressure cooking & Milling; BM - Blanching & Milling; PM - Pressure cooking & Milling; SPM - Soaking, Pressure cooking & Milling; SM - Soaking & Milling; SFM - Soaking, Fermenting & Milling; SFGM - Soaking, Fermenting, Germinating & Milling, SGKM - Soaking, Germinating, Kilning & Milling. All values are mean of triplicates with SEM and are significant if the column not sharing same alphabets in the table.

Table 5: Antioxidant Activity of Raw and Processed Horse Gram

Process	Radical Scavenging Activity (IC_{50} in µg/ml)	Reducing Power (OD_{700}) (5 µg GAE)
M (control)	0.911 ± 0.013^{b}	1.25 ± 0.004^{c}
RM	1.91 ± 0.011^{b}	0.79 ± 0.005^{a}
RPM	2.1 ± 0.013^{b}	0.72 ± 0.003^{a}
BM	1.46 ± 0.010^{b}	0.91 ± 0.007^{c}
PM	1.3 ± 0.009^{b}	0.99 ± 0.006^{c}
SPM	3.1 ± 0.012^{b}	0.36 ± 0.008^{a}
SM	0.9 ± 0.013^{c}	1.11 ± 0.004^{c}
SFM	1.8 ± 0.012^{b}	0.90 ± 0.004^{c}
SFGM	1.8 ± 0.015^{b}	0.89 ± 0.006^{a}
SGKM	1.4 ± 0.008^{b}	0.972 ± 0.005^{c}

Note: Abbreviations: M - Milling; RM - Roasting & Milling; RPM - Roasting, Pressure cooking & Milling; BM - Blanching & Milling; PM - Pressure cooking & Milling; SPM - Soaking, Pressure cooking & Milling; SM - Soaking & Milling; SFM - Soaking, Fermenting & Milling; SFGM - Soaking, Fermenting, Germinating & Milling, SGKM - Soaking, Germinating, Kilning & Milling. All values are mean of triplicates with SEM and are significant if the column not sharing same alphabets in the table.

analysed and the results are given in table 5. Highest IC_{50} value of 0.911 mg/ml was in raw sample followed by 0.9 mg/ml in SM. Reducing activity was reduced in all the processed samples when compared to control sample (1.248 OD_{700}) and slight reduction was in SM sample. Hence the results obtained on antioxidant activities were insignificant in the present investigation.

CONCLUSION

The presence of a tuft of antinutritional factors makes it primarily important to subject horse gram to several processes to bring about desirable changes in the bioavailability of nutrients. Although the toxic effects of most antinutritional factors present in plant food can be generally eliminated by suitable heat treatment, their complete destruction may not be achieved. Hence, subjecting horse gram to combination of processes may help its employability in various composite food formulations that facilitates to combat malnourishment. In the present investigation, an overall improvement in the nutritive value was observed in differently processed horse gram. Each process brought its own beneficial changes in the levels of nutrients and antinutritional factors. However, significant improvement was obtained in fermented and germinated samples in combination with soaking and pressure cooking.

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