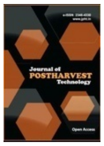

Journal of Postharvest Technology
2022, **10**(2): 48-59
www.jpht.in

RESEARCH ARTICLE

# Effect of processing treatments on nutritional, anti-nutritional and bioactive characteristics of horse gram (*Macrotyloma uniflorum* L.)

**Qurat Ul Eain Hyder Rizvi[1], Krishan Kumar[1*], Naseer Ahmed[1], Divya Chauhan[1], Priyanka Thakur[1], Sumaira Jan[1], Imran Sheikh[2]**

[1]Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, HP-173101, India

[2]Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, HP-173101, India

Received: 09.02.2022 Accepted: 11.04.2022

## ABSTRACT

Horse gram (*Macrotyloma uniflorum* L.) is an important and one of the most nutritious crops that can be utilized as a basic ingredient for the preparation of several foods because of its high nutritional value. Cereal sprouts are supposed to have a better nutritional profile than cereal grains and their products. The present study aimed to explore the changes occurring during the processing treatments such as soaking and germination of horse gram seeds. The effect of processing treatments on nutritional, anti-nutritional, minerals (Fe, Zn, Mn, and Cu), and bioactive components of horse gram was studied at an interval of 12 and 24 h during soaking and 24, 48, and 72 h during the germination treatment. The results revealed that there was a 20.66 and 23.01% rise in protein content during soaking and germination treatments, respectively. The phenolic components were enhanced to 28.49% and antioxidant activity increased by 31.51% respectively after soaking and germination treatments. The anti-nutritional components like phytic acid, trypsin inhibitor, and tannin contents decreased significantly ($p \leq 0.05$) to 40.50, 28.57, and 26.79%, respectively after 72 h of germination. The mineral contents of horse gram increased significantly after soaking and germination processing treatments.

**Keywords:** Anti-nutrients, bioactive components, germination, horse gram, soaking

**Citation:** Rizvi, Q.E.H., Kumar, K., Ahmed, N., Chauhan, D., Thakur, P., Jan, S., and Sheikh, I. 2022. Effect of processing treatments on nutritional, anti-nutritional, and bioactive characteristics of horse gram (*Macrotyloma uniflorum* L.). *Journal of Postharvest Technology*, **10**(2): 48-59.

## INTRODUCTION

India is considered as the top producer and consumer of pulses in the world. Pulses should be incorporated into the diet as it is an important source of protein for vegetarian people. Underutilized pulses are a valuable food source with numerous nutritional and health benefits for consumers and contribute effectively to global and regional food security. Among various

* *For correspondence*: K. Kumar (Email: krishankumar02007@gmail.com)

**ISSN: 2348-4330**

underutilized pulses, horse gram has gained importance because it is grown in adverse climatic conditions and is used as traditional, folk, and alternative medicine, and a potential healing agent to treat piles, common cold, fever, kidney stones, etc. It contains a high amount of proteins, fiber, and minerals such as iron, zinc, copper, and B-complex vitamins. The saturated fat content present in the horse gram is minimal (Rajagopal et al., 2017). It is a small legume crop grown widely in India and possesses a high nutritional value (Pal et al., 2016). It belongs to the family Fabaceae and has potential nutritional and remedial properties with better climate resilience to adapt to harsh environmental conditions. It is one of the most important underexploited food legumes being grown almost all over the world. Horse gram is normally considered a poor man's pulse as it offers a relatively cheap source of proteins for human consumption and livestock production. It is consumed because of its antioxidant, diuretic, and astringent nature. It is also utilized to treat various diseases like diarrhoea, haemorrhoids, kidney stones, leucorrhoea, hypertension, and gall stones (Prasad and Singh, 2015). Soaking is a domestic treatment used for the hydration of grains in water for a few hours (Embaby, 2010). Thus, it is quite useful in decreasing and eliminating the anti-nutritional components present in the food grains (Singh et al., 2017). It has been reported from various studies that soaking for 12–18 h is the most effective treatment to reduce the levels of anti-nutritional components such as proteolytic enzyme inhibitors as well as phytic acid which are partly or wholly solubilized in soaked water (Embaby, 2010; Kajihausa et al., 2014). Germination is a commonly used conventional technique that enhances the digestibility of nutrients, improves bioactive components, and reduces some anti-nutritional components in pulses. It also enhances the concentration of bioactive components like phenolic components, reducing power, metal chelating activity, flavonoids, etc. and these changes can vary depending on the variety of the seeds and the conditions of germination (Sangronis and Machado, 2007). Soaking and germination also increase the bioavailability of minerals by reducing the anti-nutritional components like phytic, tannin, and saponins that are responsible for the binding of micro and macronutrients making them unavailable to our body (Thakur et al., 2021). Soaking of dried beans for several hours brings them back to life, activating vitamins, enzymes, minerals, and proteins which have been shown to improve the free radical scavenging properties and can be used in the formulation of functional foods in our diet (Dwivedi et al., 2015). While searching for new sources of functional foods, sprouts have gained special attention throughout the world and are more often used in human diets. Hence, the sprouts may thus become a potential source of nutritious food (Shah et al., 2011). In food applications, flours prepared from the germinated yellow pea, lentil, and faba bean have better nutritional value than the raw seed. Horse gram is relatively high in iron, but the availability of the iron is reduced by the phytates, tannins, and oxalic acid. Traditional processing methods of legumes such as germination, soaking, and dehulling are sometimes used to reduce or eliminate the anti-nutrients that affect mineral and protein utilization (Vidal-Valverde et al., 2002) as well as, increase the functionality of the seeds due to the subsequent increase in the bio-active compounds (Frias et al., 2002). The current manuscript describes the effect of processing treatments i.e. soaking and germination on the nutritional, anti-nutritional, and bioactive components of horse gram to enhance the nutritional composition of products to be prepared from these processed grains.

## MATERIALS AND METHODS

### Procurement of raw materials

The horse gram (HPKM-150) used in the current study were procured from CSK Palampur. Himachal Pradesh. The chemicals used in the present study were of ultrapure grade and procured from standard manufacturers like Qualigens, BDH chemicals, Sigma, Merck India, and Hi-Media.

### Physicochemical evaluation

Physico-chemical evaluation of raw, soaked and germinated horse gram grains was carried out at laboratories of the Department of Food Technology, Eternal University located at Sirmour, Himachal Pradesh, India.

### Physical and functional parameters

The various physical parameters such as thousand-grain weights (TGW) were determined as per the method described by AACC (2000). It was expressed as the weight of thousand grains in g. The Vernier caliper was used to measure the thickness, length, and breadth and was expressed in mm. Bulk density (BD) and tap density were determined by the method of Huang et al. (2005), and Jones et al. (2000), respectively. Whereas, the water absorption capacity (WAC) and Oil absorption capacity (OAC) was determined using the methods given by Sosulski (1962) and Kaur et al. (2015), respectively. The water solubility index (WSI) was calculated using the method given by Stojceska et al. (2008).

### Chemical parameters

The air-oven drying method of AOAC (1990) was used to determine the moisture content. The Fibroplus FBS 08P (Pelican Inc.) was used to calculate the crude fiber content. Crude protein contents were estimated by Kjeloplus Kjelodist CAS VA (Pelican Inc.), Soxoplus SPS 06 AS (Pelican Inc.) was used to determine the crude fat and ash contents, as per the method described by Ranganna (1986). The total carbohydrate contents of the samples were determined by difference and calculated by subtracting the measured protein, crude fiber, crude fat, ash content, and moisture content from 100. The calorific value was determined based on the contents of fats, crude protein (N × 6.25), and carbohydrates using the Atwater factors of 9.10, 4.0, and 4.2 KCal/g of each component, respectively (WHO, 1973). Minerals were determined as per AOAC (1990) using an atomic absorption spectrometer (AA240FS, Agilent Technology, CA, USA) and were expressed in ppm. Trypsin inhibitor activity (TIA) was determined by the method of Kakade et al. (1974). The antioxidant activity (%) was evaluated according to the method defined by Bouaziz et al. (2008), and tannins were determined as per the method of Saxena et al. (2013). The quantification and extraction of biofortified wheat were assessed by the method of Gao et al., (2007), with minor modifications. Total Phenolic contents (mg GAE/100g) were determined using Folin-Ciocalteu reagent as per the method of Ainsworth and Gillespie (2007).

### Processing treatments

The soaking and germination of grains were done by the method used by Egli et al. (2002)). Clean horse gram was washed and soaked in distilled water at a 1:5 ratio. Soaking was carried out at room temperature for 12 (S12) and 24 h (S24), followed by oven drying at 40°C for 24 h. For further analysis, the soaked and dried grains were packaged and refrigerated at 4°C. The germination treatment was carried out by soaking the grains and soaked grains were then subjected to germination treatments, which were divided into numerous groups based on germination treatments. For each treatment 30g seeds were washed and steeped in 120 ml water in a beaker covered with muslin cloth for 16 hours at room temperature. The immersed seeds were drained and wrapped with damp muslin fabric. Germination took place in an incubator at 25°C for 24 (G24), 48 (G42), and 72 h (G72). During germination, the seeds were sprayed with water to keep the muslin cloth moist. After each treatment, seeds were dried for 24 hours at 40°C in a hot air oven. The grains that had been germinated and dried were packed and stored in air-tight sealable packets at 4°C until further analysis.

### Statistical Analysis

Data was assessed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics 26 software. Values in tables were stated as mean ± Standard Deviation and differences were considered significant at the level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Physical and functional and optical characteristics of horse gram

The physical and functional properties such as length, width, thickness, thousand-grain weight, etc. are presented in Table 1. The length, width, and thickness as observed in horse gram seeds were 5.23, 3.48, and 2.64 mm, respectively. Similar results were found by Patil and Kasturiba (2018) who reported 5.57 mm of length and 3.5 mm of width in horse gram. The water and oil absorption capacity found in horse gram was 1.24 and 1.90 ml/g, respectively. Ojha et al. (2020) reported 1.17 and 1.96 ml/g of water and oil absorption capacity in horse gram. The thousand-grain weight of horse gram was observed as 32.10 g and similar results were found by Vashishth et al. (2020) where thousand-grain weight was reported as 30.32 g. The bulk and tap density observed in horse gram were 0.88 and 0.97 g/cm$^3$. Bhokre et al. (2015) reported 0.74 g/ml of bulk density in horse gram. The swelling capacity observed in horse gram was 2.84% which is in accordance with the findings of Vandarkuzhali (2016) who reported a 2.29% of swelling capacity in horse gram. The optical or color characteristics determined in the form of L*, a*, and b* values in horse gram were 88.57, 3.12, and 12.98, respectively. Similar results were found by Vandarkuzhali (2016) who observed 82.34, 2.29, and 11.69 of L*, a*, and b* values in horse gram.

**Table 1. Physical, functional, and optical characteristics of horse gram**

| Parameters | Values |
|---|---|
| Length (mm) | 5.23±0.12 |
| Width (mm) | 3.48±0.15 |
| Thickness (mm) | 2.64±0.02 |
| Thousand grain weight (g) | 32.10±0.10 |
| Bulk density (g/cm$^3$) | 0.88±0.03 |
| Tap density (g/cm$^3$) | 0.97±0.02 |
| Water absorption capacity (ml/g) | 1.24±0.03 |
| Oil absorption capacity (ml/g) | 1.90±0.07 |
| Swelling capacity (%) | 2.84±0.21 |
| L* | 88.57±0.07 |
| a* | 3.12 ±0.07 |
| b* | 12.98±0.03 |

### Chemical parameters of horse gram

The result of proximate composition shows that horse gram is a good source of protein, fiber, and ash contents, and is low in fat content (Table 2). The moisture, protein, fat, ash, and fiber contents observed were 6.50, 20.66, 3.08, 3.47, and 4.45%, respectively. Similar results were found by Kachave et al. (2020) who reported 6.90, 22.50, 1.60, 2.89, and 3.17% of moisture, protein, fat, ash, and fiber contents in horse gram. The carbohydrate and calorific value found in the horse gram was 61.84% and 370.41 Kcal/100g. The phytic and tannin contents found in horse gram were 10.37 and 2.31 mg/100g, and similar results were observed by Dwivedi et al. (2015) who reported 10.4 and 2.4 mg/100g of phytic and tannin in horse gram. The antioxidant and flavonoid content found in horse gram was 20.30 and 7.70%, and similar results were found by Sreerama et al. (2012) who reported 8.63% of flavonoid content in horse gram. The in-vitro protein digestibility in horse gram was found as 77.32%, and similar results were found by Bhokre et al. (2015) who reported 79.0% of in-vitro protein digestibility in horse gram. The copper, iron, zinc, and manganese content observed in horse gram were 8.65, 16.66, 4.51, and 2.14. Whereas, Borhade et al. (1984) reported 5.5, 11.9, 3.4, and 1.5 ppm of copper, iron, zinc and manganese content in horse gram. The phenolic content found in horse gram was 140.21 mg GAE/100g, and similar results were found by Handa et al. (2017) who reported 134.7 mg GAE/100g of phenolic contents in horse gram.

**Table 2. Physical and functional characteristics of raw horse gram**

| Parameters | Values |
|---|---|
| Moisture (%) | 6.50±0.09 |
| Protein (%) | 20.66±0.48 |
| Ash (%) | 3.47±0.06 |
| Fat (%) | 3.08±0.04 |
| Fiber (%) | 4.45±0.05 |
| Carbohydrate (%) | 61.84±0.62 |
| Calorific value (kcal/100g) | 370.41±1.10 |
| Phytic (mg/100g) | 10.37± 0.06 |
| Tannin (mg/100g) | 2.31±0.01 |
| Trypsin inhibitor (mg TIA/100g) | 12.18±0.04 |
| Antioxidant activity (% inhibition) | 20.30±0.23 |
| Phenolic content (mg GAE/100g) | 140.21±0.11 |
| Flavonoid content (%) | 7.70±0.07 |
| In-vitro protein digestibility (%) | 77.32±0.69 |

### Changes in nutritional, anti-nutritional, and bioactive components of horse gram during processing treatments

#### Nutritional, bioactive components and in-vitro protein digestibility

The changes in different nutritional components and in-vitro protein digestibility are given in Table 3. The moisture content in horse gram increased from 6.50 (RG) to 7.75% after germination for 72 h. There was a 19.23% increase in moisture content after 72 h of germination treatment. The results obtained were in agreement with the findings of Kachave et al. (2020) who reported a 10.14% increase in moisture content of horse gram. Similarly, Rizvi et al. (2022) also found 24.35% increase in moisture content of pigeon pea after germination treatment. The protein content increased from 20.66 (RG) to 23.01% after germination for 72 h resulting in an 11.37% increase in protein content. Kachave et al. (2020) reported a 7.11% increase in protein contents after germination. The increase in protein content during soaking and germination might be due to the biological breakdown of various complex compounds into simpler compounds and the biosynthesis of proteins.

Ash and fiber contents increased from 3.46 (RG) to 3.57% and 4.45 (RG) to 5.90%, respectively after G72 treatment. There was 2.88% and 32.58% increase in ash and fiber content after 72 h of germination treatment. Similar results were found by Devi et al. (2015) who reported a 30.46% increase in fiber content in cowpea during germination. The crude fiber consisting of lignin, cellulose, and hemicelluloses enhanced significantly during the soaking and germination processing (Laxmi et al., 2015) due to the synthesis of different cellular compounds by the plant cells. The rise in fiber is required because dietary fiber decreases the release of glucose from food and is helpful for persons suffering from hyperglycemia (Nkhata et al., 2018).

**Table 3. Changes in nutritional, bioactive characteristics, and in-vitro protein digestibility of horse gram after processing treatments**

| **Parameters** | **Raw grains** | **Soaking (h)** | | **Germination (h)** | | |
|---|---|---|---|---|---|---|
| | | **S 12** | **S24** | **G24** | **G 48** | **G72** |
| Moisture (%) | 6.50±0.09$^{f}$ | 6.75±0.07$^{e}$ | 6.90±0.08$^{d}$ | 7.28±0.05$^{c}$ | 7.45±0.04$^{b}$ | 7.75±0.03$^{a}$ |
| Protein content (%) | 20.66±0.48$^{d}$ | 20.97±0.03$^{d}$ | 21.12±0.01$^{cd}$ | 21.63±0.07$^{c}$ | 22.84±0.02$^{b}$ | 23.01±0.59$^{a}$ |
| Fat (%) | 3.08±0.04$^{a}$ | 2.91±0.02$^{b}$ | 2.70±0.07$^{c}$ | 2.17±0.05$^{d}$ | 2.03±0.02$^{e}$ | 1.85±0.03$^{f}$ |
| Ash (%) | 3.47±0.06$^{f}$ | 3.48±0.09$^{e}$ | 3.50±0.04$^{d}$ | 3.51±0.03$^{c}$ | 3.55±0.04$^{b}$ | 3.57± 0.02$^{a}$ |
| Fiber (%) | 4.45±0.05$^{f}$ | 4.90±0.08$^{e}$ | 5.17±0.05$^{d}$ | 5.37±0.05$^{c}$ | 5.58±0.07$^{b}$ | 5.90±0.02$^{a}$ |
| Carbohydrate (%) | 61.84±0.62$^{a}$ | 60.99±0.25$^{b}$ | 60.61±0.16$^{c}$ | 60.05±0.04$^{d}$ | 58.55±0.08$^{e}$ | 57.91±0.58$^{f}$ |
| Calorific value (Kcal/100g) | 370.41±1.10$^{a}$ | 366.49±0.90$^{b}$ | 363.62±0.38$^{c}$ | 358.41±0.40$^{d}$ | 355.75±1.19$^{e}$ | 352.11±0.20$^{f}$ |
| Antioxidant activity (%) | 20.30±0.23$^{f}$ | 21.67±0.30$^{e}$ | 22.71± 0.23$^{d}$ | 25.73± 0.27$^{c}$ | 27.54±0.19$^{b}$ | 29.64± 0.29$^{a}$ |
| Phenols (mg GAE/100g) | 140.21±0.11$^{e}$ | 155.96±5.75$^{d}$ | 160.07±0.08$^{d}$ | 175.67±0.05$^{c}$ | 180.71±2.26$^{b}$ | 196.09±2.89$^{a}$ |
| Flavonoid content (mg RE/100g) | 7.70±0.07$^{f}$ | 8.31±0.06$^{e}$ | 8.66±0.03$^{d}$ | 9.30±0.08$^{c}$ | 9.61±0.02$^{b}$ | 9.92±0.09$^{a}$ |
| In-vitro protein digestibility (%) | 77.32±0.69$^{f}$ | 79.62±0.22$^{e}$ | 81.13±0.05$^{d}$ | 83.26±0.16$^{c}$ | 85.19±0.05$^{b}$ | 86.10±0.09$^{a}$ |

Values in the table are presented as mean±SD; Values within rows sharing the same letters are not significantly different according to Duncan's LSD post hoc analysis at p≤0.05

The fat content declined from 3.08 (RG) to 1.85% (germinated grains) resulting in a 39.93% reduction in the fat content of germinated grains. Whereas, carbohydrates declined from 61.84 (RG) to 57.91% (germinated grains) resulting in a 1.98 and 6.35% reduction after soaking and germination treatments. The calorific value decreased from 370.41 (RG) to 352.11 kcal/100g (G72), resulting in a 1.83 and 4.94% decrease after soaking and germination treatments.

Antioxidant activity and total phenolic components (TPC) are important characteristics of pulses that protect the cells against free radicals and play a great role in reducing the risk of various lifestyle diseases. The antioxidant and phenolic content increased from 20.30 (RG) to 29.64% (G72) and 140.21 (RG) to 196.09 mg GAE/100g (G72), respectively after germination for 72 h. The total increment in antioxidant activity was 10.61 and 31.51%, respectively after soaking and germination treatments. Whereas, the total increases in TPC was 14.16 and 39.85%, respectively after soaking and germination processing treatments. Pal et al. (2016) reported an 18.72% increase in antioxidant content after germination. Whereas, Dueñas et al. (2009) found a 60.56% increase in the antioxidant activity of lupin seeds after 5 days of germination. Khang et al. (2016) reported a 30.13% increase in TPC in adzuki beans.

The flavonoids have been recognized in various plant-based foods as an essential secondary metabolites to diminish the risk of chronic diseases like cancer and hyperlipidemia (Chen et al., 2014). The flavonoid content in horse gram increased from 7.70 (RG) to 9.92 (mg RE/100g) after 72 h of germination. There was a 28.83% increase in flavonoid contents after germination and similar results were found by Ojha et al. (2020) who reported a 30.7% increase in flavonoid content of horse gram after germination.

The in-vitro protein digestibility (IVPD) increased from 77.32 (raw grains) to 86.10% in germinated horse gram seeds. There was a 4.69 and 11.35% increment in IVPD after soaking and germination processes, respectively. Satwadhar et al. (1981) reported a 6.57% increase in IVPD in horse gram. The increase in protein digestibility might be due to the hydrolysis of protein during germination which accounts for the better digestibility of proteins (Subbulakshmi et al., 1976). The improvement of IVPD in all the processed seeds might not only be due to the decrease or removal of anti-nutrients but also due to the disintegration in the structure of the native protein, like lectins and enzyme inhibitors, breakdown of phytic acid in the seeds and the growth of endogenous α-galactosidase activity to decrease oligosaccharides (Kalpanadevi and Mohan, 2013).

## Anti-nutritional components

The phytic and tannin content in horse gram decreased from (RG) 10.37 to 6.17 mg/100g (G72) and 2.31 (RG) to 1.65 mg/100g (G72) (Table 4). The total reduction in phytic and tannin content was found to be 40.50 and 28.57%, respectively. The results obtained in the current study were comparable with the findings of Pagar et al. (2021) who reported a 39.66 and 24.77% of reduction in phytic and tannin content in germinated horse gram. The decrease in tannin and phytic acid during germination and soaking may be due to the activation of enzymes in germinated seeds, which further breaks the proteins, fats, and starches into less complex structures (Vandarkuzhali et al., 2006). Further, the reduction in tannin and phytic content was recorded higher with an increase in soaking and germination time (Handa et al., 2017).

**Table 4. Changes in anti-nutritional and mineral components of horse gram after processing treatments**

| Parameters | Raw grain | Soaking | | Germination (h) | | |
|---|---|---|---|---|---|---|
| | | S 12 | S24 | G24 | G 48 | G72 |
| Phytic acid (mg/100g) | 10.37±0.06$^{a}$ | 9.77± 0.16$^{b}$ | 9.05± 0.06$^{c}$ | 8.42± 0.09$^{d}$ | 7.45± 0.23$^{e}$ | 6.17± 0.06$^{f}$ |
| Tannins (mg/100g) | 2.31±0.01$^{a}$ | 2.25±0.04$^{b}$ | 2.08±0.03$^{c}$ | 1.88± 0.06$^{d}$ | 1.73±0.02$^{e}$ | 1.65±0.04$^{f}$ |
| Trypsin inhibitor Activity (mg TIA/100g) | 12.18±0.04$^{a}$ | 11.92±0.06$^{b}$ | 11.66±0.11$^{c}$ | 10.93±0.05$^{d}$ | 10.37±0.17$^{e}$ | 9.64±0.21$^{f}$ |
| Iron (ppm) | 16.66±0.04$^{f}$ | 16.82±0.06$^{e}$ | 17.04±0.11$^{d}$ | 17.48±0.07$^{c}$ | 17.88±0.10$^{b}$ | 18.41±0.06$^{a}$ |
| Zinc (ppm) | 4.51±0.08$^{f}$ | 4.75±0.04$^{e}$ | 4.96±0.04$^{d}$ | 5.25±0.06$^{c}$ | 5.62±0.12$^{b}$ | 5.85±0.07$^{a}$ |
| Manganese (ppm) | 2.14±0.03$^{f}$ | 2.25±0.04$^{e}$ | 2.47±0.02$^{d}$ | 2.68±0.02$^{c}$ | 2.76±0.04$^{b}$ | 2.84±0.01$^{a}$ |
| Copper (ppm) | 8.65±0.03$^{d}$ | 8.75±0.03$^{d}$ | 9.26±0.03$^{cd}$ | 9.85±0.03$^{c}$ | 11.53±0.98$^{b}$ | 12.42±0.58$^{a}$ |

Values in the table are presented as mean±SD; Values within rows sharing the same letters are not significantly different according to Duncan's LSD post hoc analysis at p≤0.05

The trypsin inhibitor activity (TIA) gets decreased during the soaking process by discarding the soaking solution after treatment. The TIA decreased from 12.18 (RG) to 9.64 after G72 treatment. There was a 4.26 and 20.85% reduction in TIA during the soaking and germination processes, respectively. Pal et al. (2016) observed a 26.79% reduction in TIA in germinated horse gram seeds. The germination decreased the TIA content and it might be due to the proteolytic activity of enzymes activated during sprouting (Chauhan and Chauhan, 2007). Zhang et al. (2015) reported significant decreases in TIA and attributed it to a consequent rise in the activity of enzyme trypsin.

### Mineral components

There was a significant increase in mineral contents after germination treatment of horse gram seeds (Table 4). The iron content in horse gram increased from 16.66 (RG) to 18.41 ppm (G72) resulting in 10.50% increase in iron content after G72 treatment and similar results were found by Pal et al. (2016) who observed a 9.95% increase in iron content after G72 treatment of horse gram. The zinc content enhanced from 4.51 (RG) to 5.85 ppm (G72), causing a 29.71% increase during soaking and germination treatments, the results obtained were comparable with that found by Chavan et al. (1989) who observed a 29.03% increase in zinc content after germination.

The copper content in horse gram increased from 8.65 to 12.42 ppm, resulting in 43.58% augmentation after G72 treatments. Similar results were found by Pal et al. (2016) who reported a 56.01% increase in copper content in horse gram. This observation is similar to other observations where it was found that germination enhances minerals and B-complex vitamins as compared to other processing techniques (El-Adawy, 2002). The manganese content increased from 2.14 (RG) to 2.84 (G72), resulting in a 32.71% rise after germination process. Similar results were found by Kajla et al. (2017) who found a 37.27% increase of Mn content in germinated flax seeds.

## CONCLUSION

The objective of the present study was to determine the impact of processing treatments like soaking and germination on nutritional composition, anti-nutritional components, and bioactive components of horse gram. The processing techniques were found to decrease the anti-nutritional properties like tannin, phytic acid, and trypsin inhibitor activity in processed grains. There was an increase in antioxidant activity and total phenolic content of germinated grains. Food security in today's world is a major concern. Protein malnutrition, which is common in India, needs to be alleviated on an urgent basis by utilization of underutilized pulses like horse gram. Food manufacturers are increasingly interested in protein foods developed from plant sources. The study found that soaking and germination of horse gram at various time intervals increased the nutritional and bioactive characteristics of germinated grains. Germination allows the modification of the protein, decreasing anti-nutritional factors and enhancing the nutritional value of flour. The flour obtained from processed horse gram can be further utilized in bakery and other food industries involved in the preparation of weaning foods for alleviation of protein deficiency in children and preventing the occurrence of lifestyle diseases among different sections of society.

## REFERENCES

AACC. 2000. Approved methods of the American association of cereal chemists (Vol. 1). Amer Assn of Cereal Chemists.

Ainsworth, E. A., and Gillespie, K. M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, 2(4): 875-877.

AOAC. 1990. Official Methods of Analysis. In (Vol. 1: pp. 684): Washington, DC.

Bhokre, C., Joshi, A., and Rodge, A. 2015. Determination of physico-chemical and functional properties of different genotypes of horse gram. Asian Journal of dairy and food research, 34(4): 307-313.

Borhade, V., Kadam, S., and Salunkhe, D. 1984. Solubilization and functional properties of moth bean (vigna aconitifolia (jacq.) marechal and horse gram (macrotyloma uniflorum (Lam.) Verdc. Proteins. Journal of Food Biochemistry, 8(3): 229-235.

Bouaziz, M., Fki, I., Jemai, H., Ayadi, M., and Sayadi, S. 2008. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry, 108(1): 253-262.

Chauhan, O., and Chauhan, G. 2007. Development of anti-nutrients free soy beverage using germinated soybean. JOURNAL OF FOOD SCIENCE AND TECHNOLOGY-MYSORE, 44(1): 62-65.

Chavan, J., Kadam, S., and Beuchat, L. R. 1989. Nutritional improvement of cereals by sprouting. Critical reviews in food science and nutrition, 28(5): 401-437.

Chen, G., Wang, H., Zhang, X., and Yang, S.-T. 2014. Nutraceuticals and functional foods in the management of hyperlipidemia. Critical reviews in food science and nutrition, 54(9): 1180-1201.

Devi, C. B., Kushwaha, A., and Kumar, A. 2015. Sprouting characteristics and associated changes in nutritional composition of cowpea (Vigna unguiculata). Journal of food science and technology, 52(10): 6821-6827.

Dueñas, M., Hernández, T., Estrella, I., and Fernández, D. 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus angustifolius L.). Food Chemistry, 117(4): 599-607.

Dwivedi, M., Vasantha, K., Sreerama, Y., Haware, D. J., Singh, R., and Sattur, A. 2015. Kaulath, a new fungal fermented food from horse gram. Journal of food science and technology, 52(12): 8371-8376.

Egli, I., Davidsson, L., Juillerat, M., Barclay, D., and Hurrell, R. 2002. The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feedin. Journal of food science, 67(9): 3484-3488.

El-Adawy, T. A. 2002. Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition, 57(1): 83-97.

Embaby, H. E.-S. 2010. Effect of heat treatments on certain antinutrients and in vitro protein digestibility of peanut and sesame seeds. Food science and technology research, 17(1): 31-38.

Frias, J., Fernandez-Orozco, R., Zielinski, H., Piskula, M., Kozlowska, H., and Vidal-Valverde, C. 2002. Effect of germination on the content of vitamins C and E of lentils. Polish Journal of Food and Nutrition Sciences, 11(SPEC; ISS 1): 76-78.

Gao, Y., Shang, C., Maroof, M. S., Biyashev, R., Grabau, E., Kwanyuen, P., Burton, J., and Buss, G. 2007. A modified colorimetric method for phytic acid analysis in soybean. Crop science, 47(5): 1797-1803.

Handa, V., Kumar, V., Panghal, A., Suri, S., and Kaur, J. 2017. Effect of soaking and germination on physicochemical and functional attributes of horsegram flour. Journal of food science and technology, 54(13): 4229-4239.

Huang, S., Shiau, C., Liu, T., Chu, C., and Hwang, D.-F. 2005. Effects of rice bran on sensory and physico-chemical properties of emulsified pork meatballs. Meat science, 70(4): 613-619.

Jones, D., Chinnaswamy, R., Tan, Y., and Hanna, M. 2000. Physiochemical properties of ready-to-eat breakfast cereals. Cereal Foods World, 45(4): 164-168.

Kachave, K., Pawar, V., and Shinde, E. 2020. Effect of germination treatment on chemical and nutritional properties of horse gram.

Kajihausa, O., Fasasi, R., and Atolagbe, Y. 2014. Effect of different soaking time and boiling on the proximate composition and functional properties of sprouted sesame seed flour. Nigerian Food Journal, 32(2): 8-15.

Kajla, P., Sharma, A., and Sood, D. R. 2017. Effect of germination on proximate principles, minerals and anti nutrients of flaxseeds. Asian Journal of Dairy and Food Research, 36(1): 52-57.

Kakade, M., Rackis, J., McGhee, J., and Puski, G. 1974. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure.

Kalpanadevi, V., and Mohan, V. 2013. Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, Vigna unguiculata (L.) Walp subsp. unguiculata. LWT-Food Science and Technology, 51(2): 455-461.

Kaur, M., Sandhu, K. S., Arora, A., and Sharma, A. 2015. Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: Physicochemical and sensory properties. LWT-Food Science and Technology, 62(1): 628-632.

Khang, D. T., Dung, T. N., Elzaawely, A. A., and Xuan, T. D. 2016. Phenolic profiles and antioxidant activity of germinated legumes. Foods, 5(2): 27.

Laxmi, G., Chaturvedi, N., and Richa, S. 2015. The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. Journal of Nutrition and Food Sciences, 5(5): 1-3.

Nkhata, S. G., Ayua, E., Kamau, E. H., and Shingiro, J. B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food science and nutrition, 6(8): 2446-2458.

Ojha, P., Bhurtel, Y., Karki, R., and Subedi, U. 2020. Processing effects on anti-nutritional factors, phytochemicals, and functional properties of horse gram (macrotyloma uniflorum) flour. Journal of microbiology, biotechnology and food sciences, 9(6): 1080-1086.

Pagar, H., Athawale, G., and Raichurkar, S. 2021. Effect of soaking, germination and drying on anti-nutrients, minerals and functional properties of horse gram along with its commercial application.

Pal, R., Bhartiya, A., ArunKumar, R., Kant, L., Aditya, J., and Bisht, J. 2016. Impact of dehulling and germination on nutrients, antinutrients, and antioxidant properties in horsegram. Journal of food science and technology, 53(1): 337-347.

Patil, A. V., and Kasturiba, B. 2018. A Study Physical Properties and Functional Characteristics of Selected Horsegram [Macrotyloma uniflorum (Lam) Verdc.] Varieties. Int. J. Curr. Microbiol. App. Sci, 7(9): 3184-3194.

Prasad, S. K., and Singh, M. K. 2015. Horse gram-an underutilized nutraceutical pulse crop: a review. Journal of food science and technology, 52(5): 2489-2499.

Rajagopal, V., Pushpan, C. K., and Antony, H. 2017. Comparative effect of horse gram and black gram on inflammatory mediators and antioxidant status. Journal of food and drug analysis, 25(4): 845-853.

Ranganna, S. 1986. Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education.

Sangronis, E., and Machado, C. 2007. Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. LWT-Food Science and Technology, 40(1): 116-120.

Satwadhar, P., Kadam, S., and Salunkhe, D. 1981. Effects of germination and cooking on polyphenols and in vitro protein digestibility of horse gram and moth bean. Plant Foods for Human Nutrition, 31(1): 71-76.

Saxena, V., Mishra, G., Saxena, A., and Vishwakarma, K. 2013. A comparative study on quantitative estimation of tannins in Terminalia chebula, Terminalia belerica, Terminalia arjuna and Saraca indica using spectrophotometer. Asian Journal of Pharmaceutical and Clinical Research, 6(3): 148-149.

Shah, S. A., Zeb, A., Masood, T., Noreen, N., Abbas, S. J., Samiullah, M., Alim, M. A., and Muhammad, A. 2011. Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. African Journal of Agricultural Research, 6(22): 5091-5098.

Singh, B., Singh, J. P., Shevkani, K., Singh, N., and Kaur, A. 2017. Bioactive constituents in pulses and their health benefits. Journal of food science and technology, 54(4): 858-870.

Sosulski, F. 1962. The centrifuge method for determining flour absorption in hard red spring wheats. Cereal Chem., 39: 344-350.

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., and Singh, V. 2012. Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry, 131(2): 462-468.

Stojceska, V., Ainsworth, P., Plunkett, A., İbanoğlu, E., and İbanoğlu, Ş. 2008. Cauliflower by-products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks. Journal of food engineering, 87(4): 554-563.

Subbulakshmi, G., Ganesh Kumar, K., and Venkataraman, L. 1976. Effect of germination on the carbohydrates, proteins, trypsin inhibitor, amylase inhibitor and hemagglutinin in horsegram and mothbean. Nutrition Reports International, 13(1): 19-31.

Thakur, P., Kumar, K., and Dhaliwal, H. S. 2021. Nutritional facts, bio-active components and processing aspects of pseudocereals: A comprehensive review. Food Bioscience: 101170.

Vandarkuzhali, N. S. 2016. Effect of germination, dehydration on physical properties of horse gram (Macrotyloma uniflorum) flour. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 10(6): 1.

Vandarkuzhali, T., Babu, A. S., and Ravichandran, C. 2006. Fundus-fovea localization image analysis based on automatic screening.

Vashishth, R., Semwal, A. D., Pal Murugan, M., Govind Raj, T., and Sharma, G. K. 2020. Engineering properties of horse gram (Macrotyloma uniflorum) varieties as a function of moisture content and structure of grain. Journal of food science and technology, 57(4): 1477-1485.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., and Kuo, Y.-H. 2002. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. European Food Research and Technology, 215(6): 472-477.

WHO. 1973. Energy and protein requirements. WHO Technical Report Series no. 522 (and FAO Nutrition Meeting Report Series no. 52. Geneva: World Health Organization).

Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., and Yang, T. 2015. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. Journal of food science, 80(5): H1111-H1119.