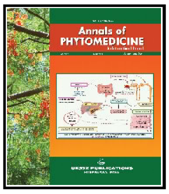
Annals of Phytomedicine 13(1): 958-965, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.103

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Processing mediated changes on protein digestibility and iron bioavailability of selected underutilized millet and legume

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Article Info

Article history
Received 4 May 2024
Revised 21 June 2024
Accepted 22 June 2024
Published Online 30 June 2024

Keywords
Barnyard millet
Germination
Horse gram
Iron bioavailability
Protein digestibility
Roasting

Abstract

The two superfoods, barnyard millet (BM) and horse gram (HG) have diversified and high nutritional value but are still underutilized and less consumed due to the presence of antinutrients which reduce the absorption and digestibility of some nutrients making them less bioavailable in human body. Traditional processing methods, *viz*., roasting and germination generally tend to improve the nutrient content as well as the digestibility of foods. The present study aimed to examine the effects of roasting and germination on protein, iron, *in vitro* protein digestibility (IVPD), and *in vitro* iron bioavailability (IVIB) of BM and HG. It was observed that the iron content, IVPD, and IVIB significantly increased after roasting and germination in BM and HG. Only the protein content decreased slightly on roasting in both BM (9.53 to 9.23%) and HG (24.94 to 23.24%), whereas germination showed a positive significant effect on protein, iron, IVPD, and IVIB of BM and HG. Consequently, the processing methods, *viz*., germination and roasting could enhance the nutritional value of the food product and hence, their consumption and utilization could be promoted.

1. Introduction

Since ancient times, millets and legumes have been consumed as staple foods in many Asian diets and cultures worldwide as they are considered as rich source of nutrients, including protein, vitamins, minerals, and antioxidants. Furthermore, because they are climate-resilient and use less water, these smart food crops satisfy all the criteria for being advantageous for the environment, the farmer, and human health due to their low carbon emissions. In Uttarakhand, mainly grown millets are barnyard millet, finger millet and mainly grown legumes are horse gram, soybean, *etc*. Consumption of millets or whole grains with pulses or legumes is associated with alleviating non-communicable diseases, as they consist of good quality protein, minerals (iron and calcium) in addition to dietary fiber, phenols, and antioxidants (Mounika and Hymavathi, 2021).

Millet is one of the ancient or oldest foods known to human beings and may have been the first cereal grain utilized for domestic purposes. The small-seeded grasses that are available all year long are referred to as millets. These grasses are quite significant in both Asia and Africa (Pathak and Singh, 2022). Barnyard millet (*Echinochloa frumentacea*) has been grown for centuries because of its great nutritional value to humans and animals, as well as its resistance to harsh environmental conditions and low input cultivation. The grains offer a variety of nutrients, including dietary fiber, minerals, other trace elements, gluten-free protein, slowly digesting starch, and phytochemicals, particularly polyphenols, and also consist of a good amount of iron (Sood *et al.,* 2015). The use of barnyard millet grains and their value-added products has consequently drawn notice all around the world. As a result, this millet has emerged as a superfood in a variety of culinary applications. In general, Indian barnyard millet is well-characterized and has 10.4% protein, bioactive components (alkaloids, tannins, and flavonoids), and mineral (iron: 15.6-18.6 mg%) content, which promote health, in comparison to other types of major food grains (Ugare *et al.,* 2014). Foods include trace amounts of bioactive molecules, and research on how these substances affect human health is ongoing (Sharma and Sarwat, 2022). Legumes are essential to human nutrition because they supply enough proteins, calories, vitamins, minerals, and other bioactive ingredients (Deshpande, 1992), and their addition to composite flour of millet or wheat could increase the protein digestibility of flours (Mounika and Hymavathi, 2021). As they are rich in amino acids like lysine and tryptophan and far less expensive than animal proteins, legumes are consumed all over the world as an alternative source of protein (Pushpangadan *et al.*, 2014). Horse gram (*Macrotyloma uniflorum*) is a leguminous crop that is grown primarily in tropical Africa and Southeast Asia and is comparatively unknown. Aside from vitamins and minerals, it is one of the underutilized legumes with high protein content. It is known as the poor man's pulse crop and is considered a great solution to combat malnutrition problems in developing nations since it provides a less

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expensive source of protein for low-income populations (Kushwaha and Raghuvanshi, 2010). It is an excellent source of protein, iron, molybdenum, and calcium (Pal *et al.,* 2016).

Although, millets and legumes have a diversified high food value and high nutritional value, their consumption, especially by the Indian populace, has not reached a significant level due to the presence of antinutrients and lack of awareness. Despite their high nutritional value and major production, these crops are being underutilized mainly because of the antinutritional factors (phytates, tannins, oxalic acid) that inhibit or reduce the absorption and digestibility of certain macro and micronutrients like protein and iron in the human body and make them less bioavailable.

Conventional processing methods such as soaking, fermentation, roasting, and germination have been used to improve the nutritional value of cereals and pulses. Roasting and germination are the two most widely employed processing methods for the preparation of food. These processing techniques are also known to reduce antinutritional compounds and trypsin inhibitors (Malomo *et al.,* 2014; Wang and Malcolmson, 2003). Processing techniques responsible for decreasing the anti-nutritional components, minimizing the losses of micro and macronutrients, and thus increasing their digestibility and bioavailability in the humans are of great interest to scientists.

VL-207 was chosen and identified due to its high yield advantage and high harvest index (25%). It has shown resistance to grain smut (6.67%), which is a common disease in barnyard millet. This high-yielding cultivar is probably going to contribute in increasing the yield of barnyard millet and ensuring food and nutritional security for the farmers.

VLG-19 with a maturity period of 88-94 days and an average yield of 5-6 q/ha, has low tannin content with good digestibility. In addition to showing a modest resistance to some diseases like collar rot, anthracnose, leaf spot diseases, and powdery mildew, and, it is resistant to root rot as well. Hill farmer's interest in the profitable cultivation of horse gram can be revived by the enhanced cultivars' superior performance and profitability as compared to local varieties.

Thus, an attempt was made in the present investigation to study the effects of roasting and germination on nutritional content and their availability in a specific variety of barnyard millet (VL-207) and horse gram (VLG-19).

2. Materials and Methods

2.1 Procurement of materials

Seeds of a selected variety of barnyard millet (VL-207) and horse gram (VLG-19) used in the current investigation were procured from the Vivekananda Parvatiya Krishi Anushandhan Sansthan (VPKAS), Almora. The seeds were properly cleaned to remove impurities and foreign material and were stored in airtight containers. Chemicals used were purchased from well-known standard companies, including Sigma, BDH Chemicals, Hi-Media, Qualigens, and Merck India.

2.2 Processing of barnyard millet and horse gram

Food processing is the process of converting raw ingredients into food or different forms of food through physical or chemical techniques. Processing methods such as roasting and germination were implemented with raw samples to test the effect of processing on protein, iron content, *in vitro* protein digestibility (IVPD), and *in vitro* iron bioavailability (IVIB).

2.2.1 Roasting of barnyard millet

Barnyard millet (BM) seeds were roasted by the method of Nithya *et al.* (2007) and Chabbra and Kaur (2022). BM seeds were roasted on a hot iron pan at 110°C for about 10 min. Then these roasted seeds were cooled and ground to flour using Bajaj Rex 750W Mixer Grinder (Pune, India) and passed through a 40 mesh size (BSS) sieve.

2.2.2 Germination of barnyard millet

BM seeds were washed thrice and then soaked in tap water (1:3 w/v) for 12 h at 27 ± 3°C. Soaked seeds were kept for germination in muslin cloth at 33°C for about 48 h. Then the germinated seeds were dried in a tray drier (Sanco, India) at 45°C for 8 h to a final moisture content of 7%, cooled, and ground to get flour using Bajaj Rex 750W Mixer Grinder (Pune, India) and passed through a 40 mesh size (BSS) sieve (Sharma *et al.,* 2016).

2.2.3 Roasting of horse gram

Horse gram (HG) seeds were roasted by the method optimized by Ojha *et al.* (2020) wherein seeds were roasted in an iron pan at 160°C for 10 min till they turned to brown colour and developed a roasted odour/smell. After letting the seeds cool, they were ground to make flour using a Bajaj Mixer Grinder, 750 W (Pune, India), and passed through a 40 mesh size (BSS) sieve.

2.2.4 Germination of horse gram

HG seeds were cleaned, washed, and soaked in tap water in a glass container (1:5 w/v) at 27 ± 3°C for 18 h. Following the removal of the soaking water, the samples were left to germinate in muslin cloth at room temperature (27 ± 3°C). Seeds were subsequently dried for 6 h at 55°C in a tray drier (Sanco, India) with up to 8% moisture content (Handa *et al.,* 2017; Banerjee *et al.,* 2022). Seeds were then cooled and ground to make flour using a Bajaj Rex 750 W Mixer Grinder (Pune, India) and passed through a 40 mesh size (BSS) sieve.

2.3 Nutritional analysis

The nutritional analysis of raw, roasted, and germinated barnyard millet and horse gram involved the determination of protein content, iron content, IVPD, and IVIB. All the estimations were performed in triplicates.

2.3.1 Protein

Protein content in the samples was determined by the AOAC (2010) method using the Micro Kjeldahl technique. The automatic nitrogen/protein estimation system (Make: Borosil, Pune, India; Model: KBD061:digestor and KDI040: distillation) was used for the estimation. The protein content was obtained by multiplying per cent nitrogen by a conversion factor of 6.25 (Jones, 1941).

2.3.2 Iron

For iron estimation, samples were wet-digested with 3:1 nitric acid: perchloric acid mixture, then cooled and filtered through Whatman No. 42 filter paper. The aliquot of each sample was used for the estimation of iron (Fe) using an atomic absorption spectrophotometer (Make: Electric Corporation of India Limited, New Delhi) by AOAC (1995) standard method.

2.3.3 *In vitro* protein digestibility (IVPD)

IVPD was estimated using the procedure given by Akeson and Stahman (1964) by slightly modifying the method of Degroot and Slump (1969) in which the sample was digested with pepsin-HCl solution followed by further digestion with pancreatin and phosphate buffer mixture. After complete digestion mixture was precipitated, centrifuged, and filtered through Whatman No.54 filter paper. The nitrogen was estimated using Micro Kjeldahl assay and converted to protein using conversion factor 6.25.

2.3.4 *In vitro* iron bioavailability (IVIB)

IVIB of samples was estimated using the method described by Rao and Prabhavati (1978), which is based on the principle that food releases ionizable iron when it is exposed to the pepsin-HCl enzyme at a pH of 1.35 and is then adjusted to 7.5 to simulate the conditions found in the stomach and intestine, respectively. After the sample was extracted at pH 1.35 using pepsin-hydrochloric acid, the pH was raised to 7.5, followed by filtering the extract through Whatman No. 44 filter paper. Ionizable iron present in the filtrate reacts with alpha-alpha dipyridyl and was determined as given in the standard method (AOAC, 1965). The ionizable iron at pH 7.5 was used as an accurate measure of bioavailable iron.

2.4 Statistical analysis

The study's findings were presented as the mean ± standard deviation of three independent replications. The statistical significance of the generated results was obtained by WASP software using one-way analysis of variance. A difference was considered significant if it was $p<0.05$.

3. Results

3.1 Effect of processing in BM

The results showing the effect of roasting and germination and percent change on nutritional composition (protein, iron, IVPD, and IVIB) of BM have been presented in Table 1 and Figure 1, respectively.

3.1.1 Protein

The protein content in the raw, roasted, and germinated BM flour ranged from 9.23 to 10.54% (Table 1) in which the protein content of germinated barnyard millet flour (10.54%) was significantly higher than raw BM (9.53%) followed by roasted BM (9.23%). An increase of 8.5% in protein content of germinated BM flour and a decrease of 6.82% in protein content of roasted BM flour was observed (Figure 1). The increase in protein content of germinated BM flour could result from a drop in dry weight because germination produces some amino acids while respiration uses some lipids and carbohydrates (Jan *et al.,* 2017; Ongol *et al.,* 2013) and the decrease in protein content of roasted BM flour was due to protein degradation (Singh and Srivastava, 2006).

3.1.2 Iron

Iron content in roasted (14.38 mg/100 g) and germinated barnyard millet (14.47%) flour was significantly higher than raw barnyard millet flour (14.18 mg/100 g) as shown in Table 1. The per cent increase in iron content of roasted and germinated BM is presented in Figure 1. Germination increases the mineral content in millets because, during this process, the phytase enzyme becomes more active, hydrolysing the link/bond between the phytic acid and the trapped minerals, releasing them and increasing their availability and content (Sharma *et al.,* 2015; Iswarya and Narayanan, 2016). The increase in iron content of roasted BM from raw BM could be because of leaching from the roasting iron pan into the millet (Obadina *et al.,* 2016).

3.1.3 *In vitro* protein digestibility (IVPD)

IVPD of roasted BM flour (85.12%) and germinated BM flour (88.14%) was found to be significantly higher than raw barnyard millet flour (74.40%) as shown in Table 1 but a non-significant difference was observed between IVPD of germinated BM flour and roasted BM flour. There was a 14.41% and 18.47% increase in IVPD of roasted BM flour and germinated BM flour, respectively from raw BM flour (Figure 1). The improvement in IVPD after germination and dry heating (roasting) could be attributed to the reduction of anti-nutrients like phytic acid, tannins, and polyphenols, which interact with proteins to form complexes. It has been observed that heat processing increases the digestibility of proteins by eliminating protease inhibitors (Abbey and Berezi, 1988; Hassan *et al.*, 2006).

3.1.4 *In vitro* iron bioavailability (IVIB)

IVIB of raw, roasted, and germinated BM flour ranged from 11.02 to 24.39% in which the IVIB of germinated BM flour (24.39%) was significantly higher than roasted BM flour (21.56%), followed by raw BM flour (11.02%) as depicted in Table 1. A significant per cent increase in IVIB of roasted BM flour (95.64%) and germinated BM flour (121.32%) was observed (Figure 1). The increase in iron bioavailability after germination might result because of the leaching of the antinutritional compounds that bind the minerals (Idris *et al.,* 2007). During germination, there is a remarkable increase in phytase activity which decreases the amount of phytic acid that binds minerals and thus leads to improved mineral accessibility and availability (Luo *et al.*, 2014).

Table 1: Effect of processing on nutritional composition of barnyard millet

Parameter	Raw BM	Roasted BM	Germinated BM
Protein	9.53 ± 0.11^{b}	9.23 ± 0.10^{c}	10.54 ± 0.11^{a}
Iron	14.18 ± 0.06^{b}	14.38 ± 0.11^{a}	14.47 ± 0.10^{a}
In vitro protein digestibility	74.40 ± 6.45^{b}	85.12 ± 6.41^{a}	88.14 ± 6.11^{a}
In vitro iron bioavailability	11.02 ± 0.87^{c}	21.56 ± 0.70^{b}	24.39 ± 0.73^{a}

Values are the mean of replications (n = 3)

^{ab} values within the row with different superscript letters differed significantly ($p<0.05$) where, a>b>c

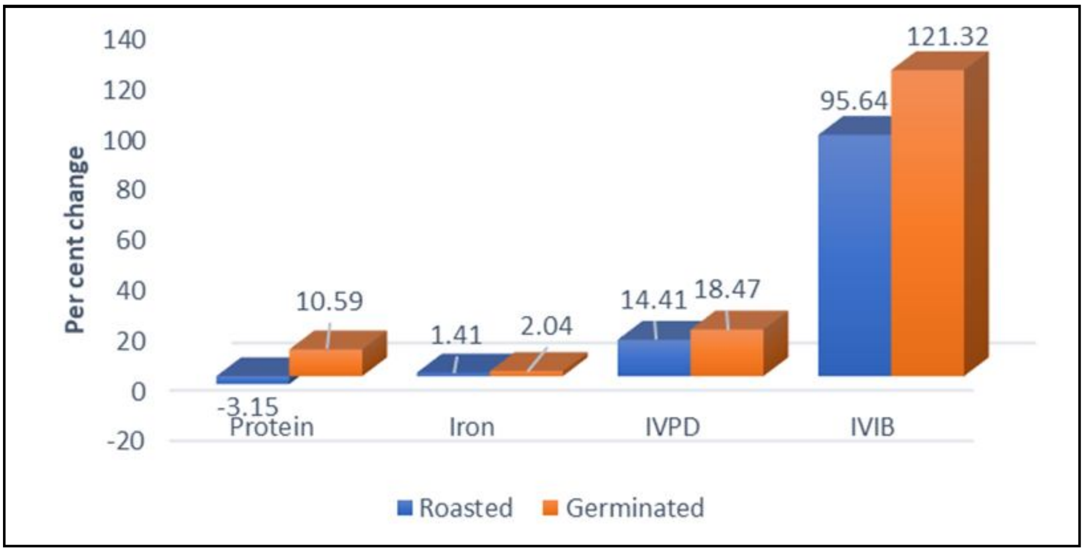

Figure 1: Per cent change in nutritional composition of BM on processing.

3.2 Effect of processing in HG

Results showing the effect of roasting and germination and per cent changes on nutritional composition (protein, iron, IVPD, and IVIB) of HG have been presented in Table 2 and Figure 2, respectively.

3.2.1 Protein

Protein content varied significantly with roasting and germination of HG as seen in Table 2. The germination showed a significant increase in the protein content from 24.94 to 27.06% whereas, roasting showed a significant reduction in the protein content of HG from 24.94 to 23.24% (Figure 2). The biological breakdown of several complex components into simpler molecules and the biosynthesis of proteins (amino acids) may be the cause of rise in the amount of protein during germination. The decline in protein content observed after roasting could be attributed to the denaturation of proteins and the release of nitrogenous volatile compounds at elevated temperatures (Wani *et al.,* 2017).

3.2.2 Iron

Iron content in raw, roasted, and germinated HG is depicted in Table 2 which showed a significant increase in iron content of roasted (9.61 mg/100 g) and germinated HG (10.98 mg/100 g) when compared with raw HG flour (9.33 mg/100 g). A 17.68% increase in iron content was seen in germinated HG flour from raw horse gram flour (Figure 2). This increase could be due to the hydrolysis of iron compounds due to high water retention in germination (Poblete *et al.,* 2020).

3.2.3 *In vitro* protein digestibility (IVPD)

IVPD of germinated HG flour (83.75%) was significantly higher than roasted (74.56%) and raw HG flour (69.79%) as depicted in Table 2. An increase of 6.83% in roasted HG and up to 20% in germinated HG flour from raw HG flour was seen (Figure 2). Protein hydrolysis and the removal of antinutrients during germination may be the cause of the increased protein digestibility (Sharma *et al.,* 2019; Ganzle, 2020). The decrease or removal of anti-nutrients, the disintegration of phytic acid and native protein structures like lectins and enzyme inhibitors, increased activity of endogenous α-galactosidase to reduce oligosaccharides might all lead to the improvement of IVPD in all processed seeds (Kalpanadevi and Mohan, 2013).

3.2.4 *In vitro* iron bioavailability (IVIB)

IVIB of raw, roasted, and germinated HG flour varied from 17.47 to 33.94% as depicted in Table 2. A significant per cent increase in IVIB of roasted (46.19%) and germinated HG flour (94.28%) from raw HG flour was observed (Figure 2). The germination process significantly increased the iron availability in horse gram from raw and roasted samples which were in agreement with the results reported by Sharma and Bhatnagar (2017). Phytic acid tends to bind cations, including calcium, iron, magnesium, and zinc. As a result of germination, the phytic acid is hydrolysed which makes these elements or minerals more bioavailable for the human body (Augustin and Klein, 1989).

Table 2: Effect of processing on nutritional composition of HG

Parameters	Raw HG	Roasted HG	Germinated HG
Protein	24.94 ± 0.29^{b}	23.24 ± 0.10^{c}	27.06 ± 0.11^{a}
Iron	9.33 ± 0.12^{c}	9.61 ± 0.09^{b}	10.98 ± 0.10^{a}
IVPD	69.79 ± 2.45^{b}	74.56 ± 2.44^{b}	83.75 ± 2.34^{a}
IVIB	17.47 ± 1.26^{c}	25.54 ± 0.70^{b}	33.94 ± 1.18^{a}

Values are the mean of replications (n = 3)

^{ab} values within the row with different superscript letters differed significantly ($p<0.05$) where, a>b>c

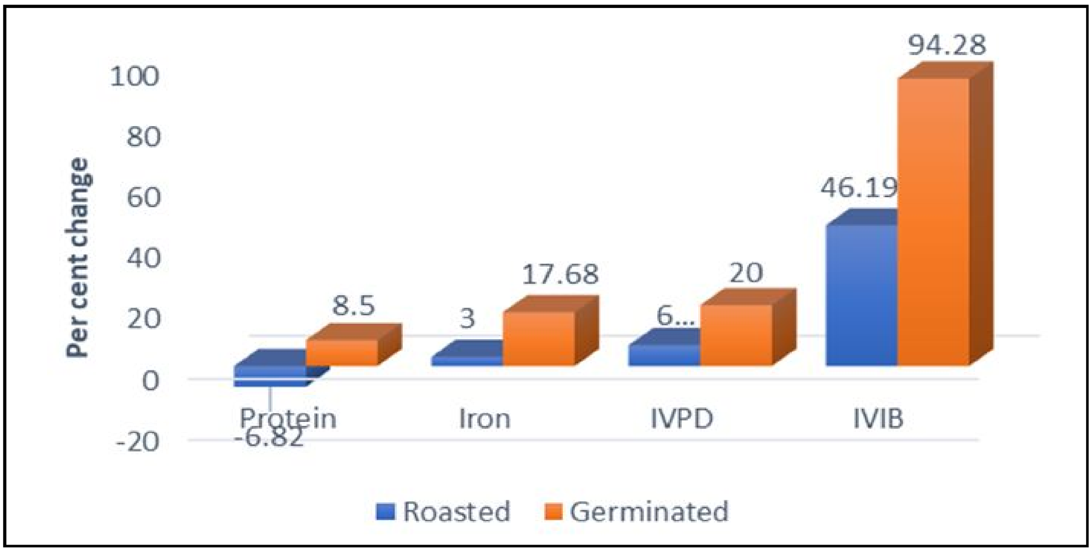

Figure 2: Per cent change in nutritional composition of HG on processing.

4. Discussion

Millets and legumes are abundant sources of protein, dietary fiber, polyphenols, and essential macro and micronutrients. Their nutritional content, digestibility, and nutrient availability are significantly affected by the methods of processing (Gowda *et al.,* 2022). Numerous studies demonstrate the substantial effects of different processing methods on the nutritional composition and the nutrient availability in BM and HG.

Kulla *et al.* (2021) observed a significant increase in the protein content of BM flour from 10.19 to 10.90% after germination. Sharma *et al.* (2016) also observed that the protein content of germinated BM (11.22%) increased significantly when compared to raw BM (9.14%) along with the iron content which increased from 37.68 to 39.42 mg/kg. These results were in contradiction with the study in which germination of sorghum was done at different time intervals, at different temperatures and reported that the protein content was negatively affected by the germination process (Raj and Singh, 2022). Nazni and Devi (2016) showed the high protein content in roasted BM flour (12.8 g) in comparison to raw (11.2 g) and germinated (8.9 g) BM flour. Contrarily, Anbalagan and Nazni (2020) observed a significant decline in the amount of protein in roasted BM (5.06%) from raw BM (5.62%). The rise in crude protein content in the sample subjected to the roasting treatment can be attributed to the use of low roasting temperatures, resulting in the generation of secondary metabolites that include protein (Mazni *et al*., 2023). Thus, low-temperature roasting increases protein content. It is recommended to roast barnyard millet at low temperatures to maximize protein availability and reduce denaturation, allowing the body to better utilize the protein.

Studies on finger millet and foxtail millet roasting showed an increase in iron content (3.45 to 3.91 mg/100 g) and (2.92 to 3.10 mg/100 g), respectively (Singh *et al.,* 2018; Khapre *et al.,* 2016) which were in contradiction with the study of Nazni and Devi (2016) who reported that the iron content of raw BM flour (9.00 mg/100 g) decreased in roasted (3.28 mg/100 g) and germinated BM flour (7.59 mg/100 g). Various researchers have reported increased protein digestibility (IVPD) of millets after a roasting or germination process (Omary *et al.,*2012; Pushparaj and Urooj, 2011). Experiments by Khetarpaul and Chauhan (1990) and Chaturvedi and Sarojini (1996) showed a notable increase up to 51 and 59%, respectively, in pearl millet protein digestibility after germination. Similarly, roasting significantly increased the IVPD of pearl millet (45.5 to 65.8% for the K variety and 49.3 to 75.4% for the MRB variety), indicating that dry heat treatment is superior to wet heat treatment in terms of improving protein digestibility (Pushparaj and Urooj, 2011). High protein content in germinated millet flours may be attributed to enzyme activation and synthesis of nitrogen-containing non-protein nucleic acids (Chethan *et al*., 2022). The high protein content, presence of free amino acids, and protein solubility in germinated millet flours can be linked to the activation of enzymes and the synthesis of proteins through biochemical events that occur during the germination process. Furthermore, it has been noted that the overall protein content of germinated grains is influenced by both the breakdown of proteins (proteolysis) and the creation of new proteins during the process of germination (Chetan *et al*., 2022).

Additionally, germination improved the bioavailable iron content of pearl millet by up to 2.2 times when compared to unprocessed grains (from 0.5 to 1.1 mg/100 g), and in finger millet, the bioavailable iron content increased from 0.4 to 1.3 mg/100 g as reported by Anitha *et al.* (2021). Thermal processing methods like roasting can improve micronutrient density in plant-based diets. Therefore, using these measures can enhance nutrient accessibility and reduce antinutrient content in food (Hotz and Gibson, 2007). Kalse *et al.* (2022) reported that roasting millet samples leached iron from the roasting pan leading to increase in their iron content.

The research carried out by Rizvi *et al.* (2022) showed that the protein content of HG increased after 72 h of germination from 20.66 to 23.01% resulting in an increase of 11.37% along with the increase in IVPD of germinated HG (86.10%) from raw HG (77.32%) which was also in agreement with the study conducted by Kachave *et al.* (2020) and Ghavidel and Prakash (2007). The rise in protein content may be attributed to protein production occurring during germination (Kachave *et al*., 2020). Roasting resulted in a slight decrease in the protein content of HG from 21.40 to 20.60% (Thirukkumar and Sindumathi, 2014). Similarly, Kumar *et al.* (2020)

also showed a decrease in the protein content of roasted chickpeas (13.00%) from the unroasted sample (14.80%). Roasting also resulted in a slight increase (3.2-15% increase) in protein digestibility (IVPD) of roasted soybeans when compared to their raw counterpart (Baik and Han, 2012; Raigar and Mishra, 2021). Various authors reported a significant increase in the iron content of germinated HG flour than roasted and raw HG flour (Rizvi *et al.,* 2022; Atudorei *et al.,* 2021; Pal *et al.,* 2016). Germination significantly increased the iron availability in HG from raw and roasted samples which were in agreement with the results reported by Sharma and Bhatnagar (2017) in which it was seen that the germination of HG increased IVIB from 26% to 37.93% and roasting of HG increased IVIB from 26% to 28.30%. Luo *et al.* (2014) reported that germination showed an enormous improvement in the iron bioavailability (IVIB) of faba bean (6.34 to 31.47%) and soybean (6.16 to 30.67%) which was a nearly fourfold increase. Our findings align with those presented by El-Adawy *et al*. (2002), who indicated that the loss of divalent metals (Ca, Fe, and Zn) during germination is minimal. This is attributed to their binding to proteins and the creation of phytate-cation-protein complexes. Similarly, Ghavidel and Prakash (2007) revealed that the percentage of bioavailable iron in chickpeas, green gram, cowpeas, and lentils increased dramatically by 64.6%, 67.8%, 75.8%, and 81.3%, respectively, over the raw counterparts after germination. However, the iron content of chickpeas and cowpeas decreased during the same period. The increase in iron bioavailability in germinated horse gram flour is attributed to the soaking and germination treatment, which leads to a reduction in phytate concentration (Sharma and Bhatnagar, 2017). Phytate is a major inhibitor of iron and zinc absorption (Sandberg, 2002).

5. Conclusion

The study demonstrates that germination and roasting significantly enhance the protein and iron content, as well as the digestibility and availability, of BM and HG flours. Empirical data show that germination notably increases nutrient content and availability compared to raw and roasted counterparts, primarily by reducing anti-nutritional factors through enzyme activation. However, future research is yet required to determine how these processing techniques affect other nutrients as well. These traditional processing methods improve nutrient bioavailability, making roasted and germinated millet or legume flours ideal for developing nutrient-rich products and therapeutic diets.

Acknowledgements

The authors thank the Department of Food Science and Nutrition, College of Community Science, GBPUAT, Pantnagar, Uttarakhand, India for helping in conducting this research.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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Citation **Ayushi Joshi, Archana Kushwaha, Anuradha Dutta, Anil Kumar and Navin Chandra Shahi (2024). Processing mediated changes on protein digestibility and iron bioavailability of selected underutilized millet and legume. Ann. Phytomed., 13(1):958-965. http://dx.doi.org/10.54085/ap.2024.13.1.103.**