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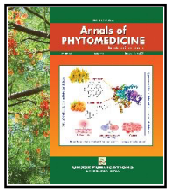

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Varietal and processing influence on nutrient retention and bioavailability of pearl millet [*Pennisetum glaucum* (L.) R. Br.]: A comprehensive analysis

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Abstract

Long-forgotten pearl millet has re-entered the market of Punjab owing to its increasing popularity associated with health-promoting benefits. Still, there is a limited understanding of exploring its nutritional aptitude and application of appropriate standardized processing treatment to enhance its nutritional potential. Therefore, the present investigation examines the influence of germination, soaking, roasting, puffing, and parling on nutrient retention and bioavailability of two pearl millet cultivars (FBC 16 and PCB 165). Germination significantly (p=0.05) improved protein content (5.52 to 6.35%), *in vitro* protein digestibility (30.2 to 60.0%), and total dietary fiber (2.9 to 16.2%), while parling as well improved protein digestibility (24.7 to 43.0%). Total iron (11.7 to 17.6%) was reduced while zinc (17.8%) and copper (3.8%) increased considerably by germination. Germination (70.9 to 73.3%) and puffing (37.5 to 64.4%) enhanced iron bioavailability with bioavailable calcium. However, germination and puffing served as an appropriate food-based strategy to gain and retain nutrients such as protein, dietary fiber, antioxidants, iron, and other minerals maximally from pearl millet as compared to other applied treatments. Hence, pearl millet grains must be projected to these treatments to improve their acceptability, and food products prepared using processed pearl millet may help in alleviating malnutrition.

1. Introduction

The dual burden of malnutrition with changing climatic conditions and the ever-growing human population demands ensuring of sustainable food and nutritional bank, a major challenge in this Anthropocene. Cereals being staple globally, the most significant crop substitutes following wheat and rice are coarse grains such as millets. Pearl millet [*Pennisetum glaucum* (L.) R. Br.], a significant crop in both Asia and Africa, possesses high-level salinity tolerance and drought resistance with significant potential for ensuring food security in developing countries, thus, capturing the attention of the researchers for its use as feed, food, and fodder, since food is essential for the body's proper development and for preserving a person's health (Pathak and Singh, 2022; Gupta and Sarwat, 2022). Nonetheless, as per the database of Punjab Agricultural University, the history of millet cultivation in Punjab revealed a richer story, since 11 lakh hectares of Punjab's land was under millet cultivation during the 1950s. However, following the onset of the Green Revolution in 1965-66, the area under millet cultivation began to decline, dropping to just 2.13 lakh hectares by 1969-70 (Chhabra and Mahajan, 2023). However, recent trends showed increased interest of consumers towards pearl millet due to its nutritional potential.

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Structurally, pearl millet has low prolamin content (gluten-free) while higher proportions of slowly digestible and resistant starch reduce its glycemic index (Martins *et al.,* 2018). Also, its protein load is considered identical or superior with essential amino acids (leucine, isoleucine, and lysine), micronutrients such as iron, zinc, and B-complex vitamins, and a high amount of soluble and insoluble dietary. Being rich sources of protein, minerals, dietary fiber, phenols, and antioxidants, consumption of millets and whole grains are associated with a reduction in the incidence of non-communicable diseases (Joshi *et al*., 2024). However, various research studies are further exploring the effects of these bioactive compounds on human health (Sharma and Sarwat, 2022). The limiting factor is the acceptance of the crop due to its coarse nature, low digestibility, presence of anti-nutritional factors, high fat content, and rapid activity of some enzymes causing huge postharvest losses (Akinola *et al.,* 2017). However, the application of processing treatments could improve its nutritive and sensory characteristics. Research evidence revealed that malting significantly ameliorated the protein (9.6%) and mineral (Mn: 11.60%, Cu: 11.03%, Ca: 66.04%, P: 46.65%) component of intact red sorghum (Omoikhoje and Obasoyo, 2018). Along similar lines, renal absorption of iron and other micronutrients of pearl millet could be peaked with appropriate processing by softening the protein matrix and releasing protein-bound iron and micronutrients, thus aiding the bioavailability of nutrients. Therefore, consumption of iron-rich value-added millet-based products can be useful in meeting most of the dietary iron requirements and can curb the widespread problem of iron deficiency anaemia (Choudhury and Chaudhary, 2023).

Despite its potential, there is limited understanding of the nutritional value of pearl millet and the impact of standardized processing

treatments on enhancing its nutritional profile. Previous laboratory studies have not thoroughly investigated the effects of multiple conventional processing techniques on the nutritional parameters of pearl millet cultivars FBC 16 and PCB 165, developed by Punjab Agricultural University, Ludhiana (India). This study aims to fill that gap by conducting a comprehensive analysis of five viable processing methods, focusing on nutrient retention and bioavailability, with the goal of standardizing processes for developing value-added flour suitable for both household and industrial use.

2. Materials and Methods

Pearl millet cultivars [FBC 16 (PM-1) and PCB 165 (PM-2)] sourced from the Seed Production Farm of Punjab Agricultural University, Ludhiana, were cleaned and subjected to the following treatments (Table 1).

Table 1: Processing of sample

Treatment	Method
Germination	Grains were soaked in water (1:2) overnight (30°) and treated with formaldehyde (0.2%) tailed by washing and incubating in muslin cloth at 30°C. Germination was done for 48 h (90-95% RH) and dried to a constant weight at 50°C in a hot air oven.
Soaking	The cleaned grains of pearl millet were soaked overnight at room temperature (25°) in excess distilled water in a dish, covered with a muslin cloth for 6 h.
Roasting	Application of dry heat at 190° and remove from the flame immediately after the roasted nutty flavour begin to arise from the pan of grains (after 3 min).
Puffing	By the method of Malleshi and Desikachar (1981), moisture content was raised to 19% and puffed in an iron frying pan using fine sand as a heat exchange medium (270°C). Puffed sample was separated by sieving through 40 mesh sieve.
Parling	Grains were parled for one minute by a mechanical method using 'Barley Parler Control Unit' with approximately 8-9% removal of bran (decortication).

Control (unprocessed grains) and processed pearl millet grains were dried in a hot air oven (Model MSW 211, Macro Scientific Works Pvt. Ltd., India) to achieve desired moisture level (5%) and then finely ground using mortar and pestle. Samples were stored in labelled, airtight containers. All chemical reagents used were of analytical grade and reagent kits were purchased from Sigma-Aldrich, Inc. Sigma Chemical Co. (USA).

2.1 Nutritional composition

Moisture (AACC, 2010) and ash (gravimetrically by charring then igniting at 550°C in a muffle furnace) (AACC, 2010) were determined. Total lipid analysis (AOAC, 1997) was carried out using SOCS plus Solvent Extraction system (Pelican, India). Protein content using the Kjeldahl method (AACC, 2010) in KEL PLUS Automatic Nitrogen System (Pelican Equipment, Chennai, India) with a factor of 6.25 applied to convert the amount of nitrogen to crude protein. Total dietary fiber was quantified using a modified enzymatic gravimetric method (AOAC, 2005).

2.2 Bioactive compounds

Extraction using cold maceration technique in methanol (1 g/ 15 ml of 80% acidified methanol). Supernatants were centrifuged at 3.500 × *g* for 15 min, filtered, volume (50 ml) with solvent, and stored at a low temperature of -20°C in microcentrifuge tubes. Total phenolic content was determined by the method described by Shen *et al.* (2018). Addition of Folin-Ciocalteu reagent (0.5 ml) and 7.5% Na_2CO_3 (10 ml), incubated (37°C for 60 min), read at 750 nm against the blank and expressed in mg equivalent of gallic acid (GAE) per 100 g dry weight. Quantitative estimation of tannins was done by a modified method of Owheruo *et al.* (2018) using Folin-Ciocalteu reagent and 2.5 ml of 20% Na_2CO. The mixture vortexed, incubated for 40 min at room temperature, and read at 725 nm. A standard curve was prepared using 0-100 µg of tannic acid. Estimation of total flavonoid content was performed using a method by Owheruo *et al.* (2018) by adding $NaNO_2$, followed by $AlCl_3$ then 1N NaOH, read at 510 nm against blank (5 ml deionized water). A standard series of known concentrations of Rutin (50-200 µg) was prepared for analysis.

2.3 Antioxidant activity

Total antioxidant activity by DPPH (1,1 diphenyl- picrylhydrazyl/ 2,2-Diphenyl-1-picrylhydrazyl) was measured following the methodology stated by Singh *et al.* (2019) by addition of 3 ml of DPPH reagent and incubation, read at 517 nm.

Per cent inhibition = [(Ac – Ae)/ Ac] × 100

where,

Ac = Absorbance of control

Ae = Absorbance of extract

TAC (mg TE/100 g) = Standard concentration/Standard per cent inhi* Sample per cent inhi / Aliquot taken* Volume made up/ Sample taken* 100/1000* Dilution factor (*Standard curve for all bioactive)

Total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay was measured according to the method by Shen *et al.* (2018) using FRAP working reagent and incubation then read at 593 nm against blank.

2.4 *In vitro* protein digestibility

According to Sharma *et al.* (2018), a sample (0.5 g) was mixed with pepsin and pancreatin solution (50 ml), and incubated for 24 h after each enzymatic addition. Samples, were centrifuged (13000 × *g* for 10 min) and filtered to analyse the nitrogen content (macro Kjeldahl method). The digestibility coefficient was calculated.

2.5 *In vitro* starch digestibility

The assay was analysed using methodology by Sharma *et al.* (2018). Use of pancreatic amylase (0.5 ml) solution (20 mg/ 50 ml phosphate buffer) followed by incubation. Quick addition of 2 ml of 3, 5 dinitrosalicyclic acid, read at 550 nm (Spectro 20D Plus RS-232, California, USA).

2.6 Minerals

Estimated using Atomic Absorption Spectrophotometer (A Analyst 200, Perkin Elmer, USA) post-wet digestion (Merwe *et al.,* 2019) using a freshly prepared di-acid mixture of nitric acid and perchloric acid (4:1) while digital clinical flame photometer (Model 882, Max electronics, India) measured the concentration of potassium and phosphorus.

Mineral content (mg) = Concentration of sample (ppm) x Dilution factor/100

2.7 *In vitro* mineral bioavailability of iron

The methodology stated by Rao and Prabhavati (1978) used pepsin-HCl solution (pH 1.35), incubated at 37!, filtered (pH 7.5), and centrifuged at 10500 x *g* for 15 min. Supernatant was determined for ionizable iron using equation:

Y = 0.4827 + 0.4707X,

where,

Y = Per cent iron absorption in adults,

X = Per cent ionizable iron at pH 7.5

2.8 *In vitro* mineral bioavailability of calcium and zinc

Solubility and dialysis tests as specified by Rebellato *et al.* (2020) by infusion of pepsin solution (0.65 ml) [1.6 g of pepsin, P-7000, (Sigma Chemical Co., St. Louis, USA)], pancreatin-bile salt mixture (6.5 ml; pH 5) [0.4 g of pancreatin, P-7545, and 2.5 g of bile salt porcine, B-8631, (Sigma Chemical Co., St. Louis, USA)], centrifugation at 10500 x *g* then subjecting to dialysis method (dialysis bag: molecular weight cut-off from 12,000 to 16,000 and porosity of 25 angstroms containing specified amount of water and $NaHCO_3$), analysed using Atomic Absorption Spectrometer:

Bioavailability (%) = 100 ×Y/Z, Y (mg mineral element/ 100 g of sample), Z (mg mineral element/ 100 g of sample).

2.9 Statistical analysis

Mean, standard deviations, one-way analysis of variance and post hoc test (Tukey's) and the means separated using Least Significant Difference at $p \leq 0.05$, performed using IBM SPSS Inc. (version 23, Chicago, USA).

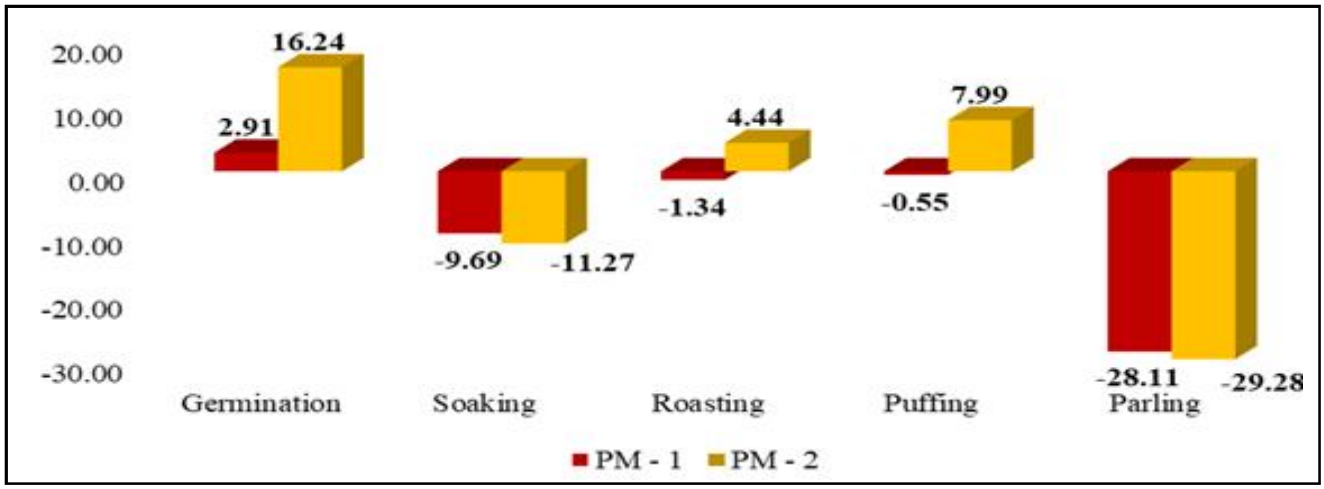

Figure 1: Per cent change in total dietary fiber content on processing.

Table 2: Effect of various treatments on the nutritional composition of pearl millet cultivars (g/100 g on dry matter basis)

Treatment		Control	Germination	Soaking	Roasting	Puffing	Parling
Moisture	PM-1	04.32 ± 0.12^{b}	06.63 ± 0.23^{a}	04.62 ± 0.37^{b}	03.68 ± 0.03^{c}	03.29 ± 0.06^{c}	04.37 ± 0.04^{b}
	PM-2	05.14 ± 0.35^{c}	11.15 ± 0.45^{a}	09.49 ± 0.45^{b}	05.13 ± 0.26^{c}	02.52 ± 0.06^{d}	04.46 ± 0.24^{c}
Ash	PM-1	02.64 ± 0.08^{a}	02.24 ± 1.17^{a}	01.52 ± 0.04^{b}	01.53 ± 0.08^{b}	02.58 ± 0.19^{a}	02.48 ± 0.17^{a}
	PM-2	03.80 ± 0.18^{b}	02.17 ± 0.03^{c}	03.23 ± 0.05^{b}	03.35 ± 0.08^{b}	07.11 ± 0.62^{a}	02.18 ± 0.03^{c}
Crude protein	PM-1	11.59 ± 0.53^{ab}	12.23 ± 1.07^{a}	10.58 ± 0.54^{ab}	11.23 ± 0.18^{ab}	11.80 ± 0.51^{a}	09.96 ± 0.81^{b}
	PM-2	11.65 ± 0.71^{a}	12.39 ± 0.16^{a}	10.33 ± 0.45^{b}	11.66 ± 0.40^{a}	11.21 ± 0.31^{ab}	10.00 ± 0.43^{b}
Crude fat	PM-1	05.00 ± 0.56^{a}	02.80 ± 1.59^{b}	03.95 ± 0.10^{ab}	03.52 ± 0.18^{ab}	03.31 ± 0.27^{ab}	02.76 ± 0.94^{b}
	PM-2	05.01 ± 1.24^{a}	03.01 ± 0.47^{bc}	04.68 ± 0.48^{ab}	04.08 ± 0.40^{ab}	03.84 ± 0.15^{abc}	02.19 ± 0. 47^{c}
Total dietary fiber	PM-1	12.70 ± 0.60^{a}	13.07 ± 0.31^{a}	11.47 ± 0.91^{a}	12.53 ± 0.70^{a}	12.63 ± 0.45^{a}	09.13 ± 0.40^{b}
	PM-2	11.27 ± 0.35^{ab}	13.10 ± 0.36^{a}	10.00 ± 1.28^{bc}	11.77 ± 0.47^{ab}	12.17 ± 1.00^{a}	07.97 ± 0.55^{c}

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters. PM-1: Pearl millet -FBC 16; PM-2: Pearl millet PCB-165.

3. Results

3.1 Nutritional composition

The crude protein varied significantly ($p \leq 0.05$) with the highest content in germinated pearl millet flours (12.23-12.4%) while fat decreased significantly ($p \leq 0.05$) post-processing particularly in parled (2.76-2.19%) and germinated (44.0-56.3%) variants due to removal of bran and germ, enveloping major proportion of lipids (Table 2). Estimation of total dietary fiber revealed a significant ($p \leq 0.05$) decrease in the content post-parling, while significantly ($p \leq 0.05$) higher content post-germinating (13.1%) and puffing (12.2%) of PM-2 was observed (Figure 1).

3.2 Bioactive compounds

Parling (15.9-19.6%), puffing (19.6-21.7%), soaking (13.6-13.8%) and roasting (3.8-20.7%) were found to reduce the total phenolic content of both the variants significantly ($p \leq 0.05$) while germination (19.3-23.4%) increased the content (Figure 2). A significant ($p \leq 0.05$) reduction of 19.4 to 19.6, 17.8 to 24.1, 9.0 to 16.4, 7.8 to 12.8, and 3.1 to 14.8 per cent was observed in the tannin content of parled, germinated, roasted, soaked, and puffed flours, respectively in both the varieties. Analysis of processed PM-1 flour pronounced that parled, roasted, soaked, and puffed (112.3, 121.1, 132.7 and 136.4 mg RE/100 g, resp.) variants had significantly ($p \leq 0.05$) lower total flavonoid content than control (165.14 mg RE/100 g) while among PM-2, reduction in the total flavonoid content of puffed, roasted, soaked and parled (131.9, 133.2, 139.5 and 108.2 mg RE/100 g, resp.) was observed.

3.3 Total antioxidant capacity by DPPH (1,1 diphenyl-picrylhydrazyl/2,2-diphenyl-1-picrylhydrazyl) and ferric reducing antioxidant power (FRAP)

In both the pearl millet cultivars, a significant ($p \leq 0.05$) rise in the activity by germination (12.4 and 16.2%) in PM-1 by DPPH and FRAP, respectively while 26.8 per cent in PM-2 by FRAP was observed. Significant ($p \leq 0.05$) reduction in the antioxidant capacity (DPPH) after subjecting to high temperatures (roasting and puffing) was also detected. A significant ($p \leq 0.05$) reduction of 19.8 and 35.3 per cent in PM-1 and 37.6 and 31.5 per cent in PM-2 in the antioxidant activity by DPPH and FRAP, respectively was observed in parled variants of both cultivars (Figure 3).

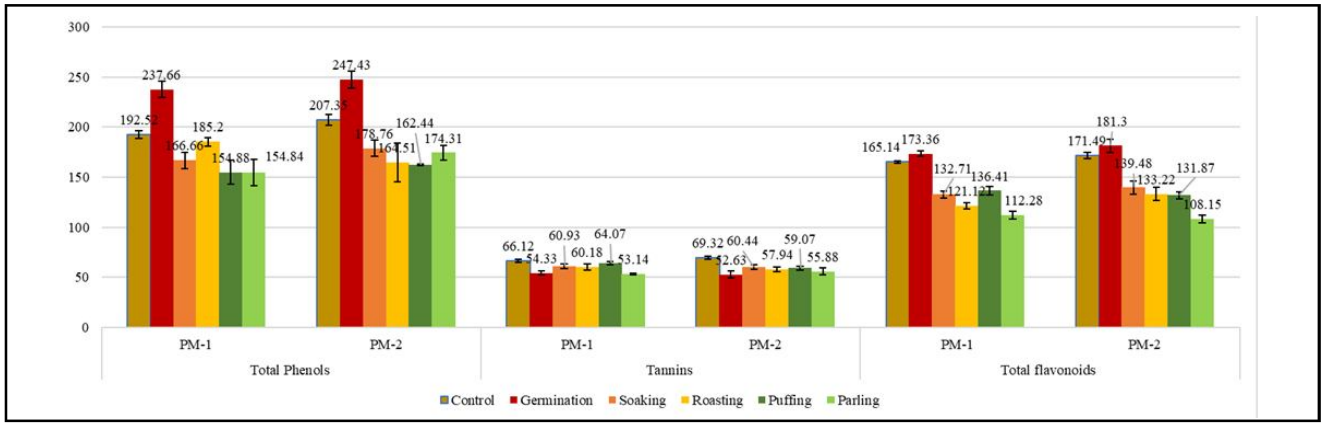

Figure 2: Effect of various treatments on bioactive compounds of pearl millet cultivars (dry matter basis).

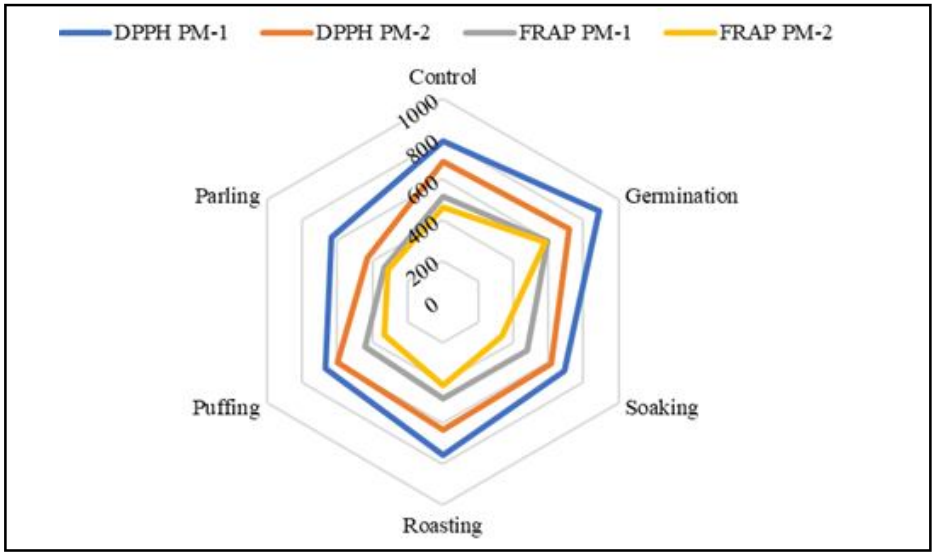

Figure 3: Effect of various treatments on antioxidant activity of pearl millet cultivars.

3.4 *In vitro* protein and starch digestibility

A significant increase ($p \leq 0.05$) in protein digestibility post processing PM-1 flour to the tune of germination (70.5%) > parling (63.04%) > puffing (50.6%) > roasting (50.3%) was observed, while an increase of 61.6 per cent was displayed by germinated tailed by soaked (48.8%), roasted (50%) and puffed (49.8%) grains of PM-2 (Figure 4). Nutritional analysis revealed that *in vitro* starch digestibility of both pearl millet cultivars was improved significantly ($p \leq 0.05$) post germination (29.5 and 27.8 mg, respectively) and parling (20.5 and 21.3 mg, respectively) (Figure 5).

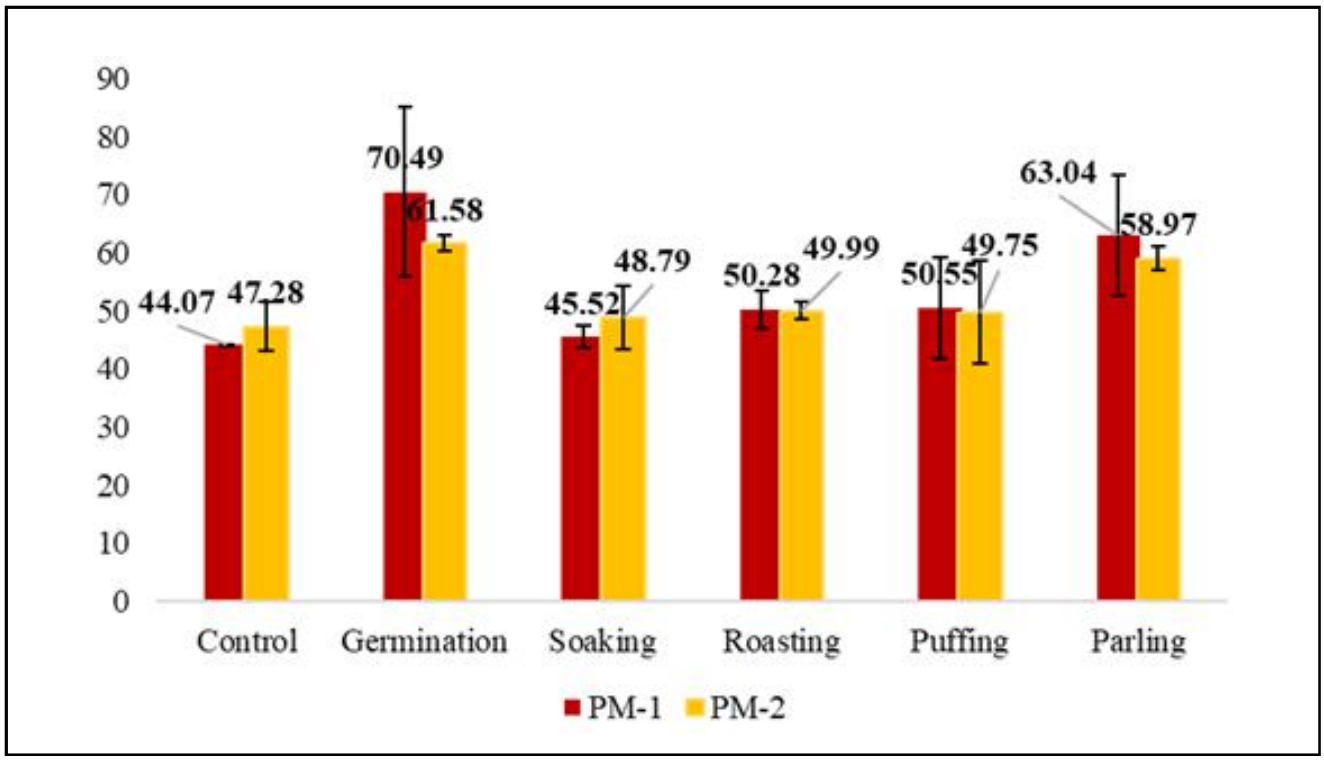

Figure 4: ***In vitro*** **protein digestibility (%) on processing.**

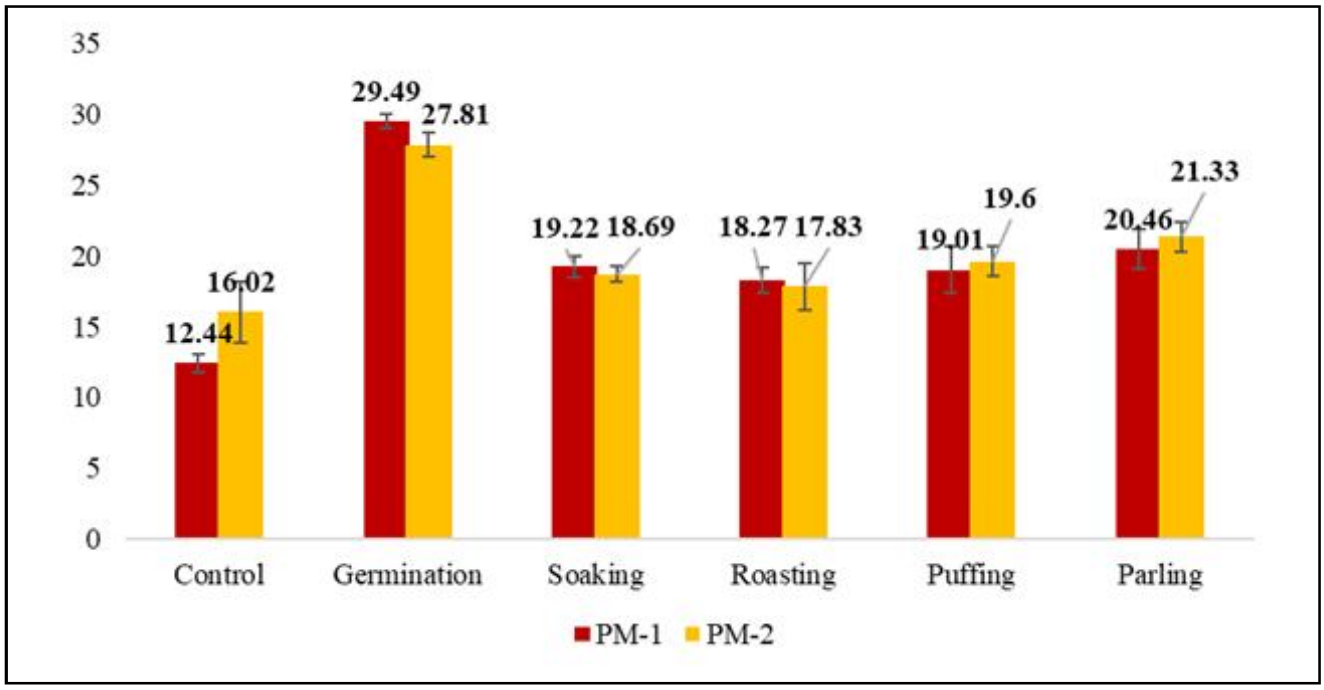

Figure 5: ***In vitro*** **starch digestibility (mg maltose released/ g) on processing.**

3.5 Minerals and their bioavailability

Analysis of the effect of processing applications on mineral content of pearl millet cultivars revealed varied results (Table 3 and 4). Significant ($p \leq 0.05$) reduction in the iron content of germinated (17.6%), parled (15.6%), and soaked (10.6%) variants (PM-1). Parling, germination, and soaking significantly ($p \leq 0.05$) reduced the iron content (PM-2) by 38.1, 11.7, and 15.6 per cent, respectively. An increase in the total calcium (17.8%) post-germinating PM-1 was observed while puffing (15.3%) and parling (34.3%) reduced the content significantly ($p \leq 0.05$). Similarly, an increase in the zinc content post-soaking (18.5%) and germination (21.6%) of PM-2 was recorded while a significant ($p \leq 0.05$) decrease in the potassium post-parling (28.3%), soaking (21.5%), germination (17.7%), roasting (13.3%) and puffing (5.7%) PM-1 and soaking, germination and parling (17.4, 20.5 and 26.3%, resp.) PM-2 was observed. Analysis of phosphorus (PM-1) revealed a reduction post germination (28.1%), soaking (22.2%), puffing (19.6%), roasting (18.8%), and parling (22.4%). Phosphorus analysis of processed PM-2 flours was comparable to that of PM-1.

Analysis of copper (PM-1) revealed a significant ($p \leq 0.05$) increase post-germination (3.8%) and soaking (1.4%) while roasting (8.3%), puffing (24.3%) and parling (49.6%) decreased the content. The magnesium content of PM-1 (12.8%) and PM-2 (12.6%) flours increased significantly ($p \leq 0.05$) post-germination. However, soaking and parling (20-21% approx.) reduced the content of both cultivars by approximately 6-12 and 20-21 per cent, respectively. Manganese content was found to be significantly ($p \leq 0.05$) lower in puffed (10%), roasted (11.8%), and parled (50.4%) variants of PM-1, whereas a notable loss of 57.8% was recorded for parled PM-2.

In vitro bioavailability of calcium, iron, and zinc increased significantly ($p \leq 0.05$) by various processing techniques. The maximum absorption of iron was observed post-germination (68.5%), followed by puffing (64.4%). Germination significantly ($p \leq 0.05$) increased the iron absorption by 71.3 per cent in PM-2 cultivar, while other processed variants followed the order of roasting > puffing > soaking (4.06, 3.59, 3.12%, resp.). Among the selected processing techniques puffed (53.5%) and parled (50.9%) variants of PM-1 displayed maximum calcium bioavailability. Zinc bioavailability (PM-1) also increased by parling (22.5%) and germination (19.4%). Similarly, higher zinc absorption was observed in parled (25.4%), puffed (23.1%), germinated (21%), roasted (18.9%), and soaked (17.4%) in PM-2 cultivars.

Table 3: Effect of various treatments on micro mineral content of pearl millet cultivars (mg/100 g on dry matter basis)

Treatment		Control	Germination	Soaking	Roasting	Puffing	Parling
Iron	PM-1	7.48 ± 0.23^{a}	6.16 ± 0.28^{b}	6.69 ± 0.28^{b}	7.36 ± 0.18^{a}	7.55 ± 0.30^{a}	6.31 ± 0.12^{b}
	PM-2	7.55 ± 0.15^{a}	6.67 ± 0.15^{b}	6.37 ± 0.13^{b}	7.30 ± 0.22^{a}	7.67 ± 0.13^{a}	4.67 ± 0.24^{c}
Zinc	PM-1	3.57 ± 0.09^{a}	3.77 ± 0.21^{a}	3.63 ± 0.18^{a}	3.59 ± 0.07^{a}	3.52 ± 0.08^{a}	2.62 ± 0.05^{b}
	PM-2	3.29 ± 0.38^{b}	4.00 ± 0.06^{a}	3.90 ± 0.19^{a}	3.29 ± 0.21^{b}	3.31 ± 0.10^{b}	2.00 ± 0.07^{c}
Copper	PM-1	0.494 ± 0.03^{ab}	0.51 ± 0.48^{a}	0.50 ± 0.07^{ab}	0.45 ± 0.05^{ab}	0.37 ± 0.05^{bc}	0.25 ± 0.04^{c}
	PM-2	0.440 ± 0.11^{ab}	0.46 ± 0.02^{ab}	0.44 ± 0.09^{ab}	0.49 ± 0.06^{a}	0.44 ± 0.04^{ab}	0.29 ± 0.03^{b}
Manganese	PM-1	1.130 ± 0.05^{a}	1.11 ± 0.24^{a}	1.09 ± 0.27^{a}	1.00 ± 0.13^{ab}	1.02 ± 0.17^{ab}	0.56 ± 0.09^{b}
	PM-2	1.160 ± 0.07^{a}	1.14 ± 0.21^{a}	1.22 ± 0.15^{a}	1.02 ± 0.22^{a}	0.97 ± 0.25^{a}	0.49 ± 0.02^{b}

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters. PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165.

Table 4: Effect of various treatments on macro mineral content of pearl millet cultivars (mg/100 g on dry matter basis)

Treatment		Control	Germination	Soaking	Roasting	Puffing	Parling
Calcium	PM-1	049.04 ± 8.59^{ab}	057.77 ± 6.66^{a}	050.39 ± 5.34^{ab}	047.87 ± 5.56^{abc}	041.54 ± 4.03^{bc}	032.20 ± 2.78^{c}
	PM-2	052.31 ± 6.87^{a}	059.27 ± 8.59^{a}	048.70 ± 3.76^{a}	049.02 ± 8.60^{a}	044.23 ± 5.45^{a}	043.26 ± 2.33^{a}
Potassium	PM-1	358.87 ± 3.73^{a}	295.21 ± 9.28^{abc}	281.87 ± 50.09^{bc}	311.25 ± 19.75^{abc}	338.40 ± 36.36^{ab}	257.32 ± 7.50^{c}
	PM-2	415.87 ± 45.79^{a}	343.42 ± 43.42^{ab}	330.56 ± 38.56^{ab}	413.44 ± 45.19^{a}	434.53 ± 34.05^{a}	306.37 ± 4.39^{b}
Phosphorus	PM-1	305.32 ± 15.94^{a}	219.60 ± 6.74^{c}	237.49 ± 3.65^{bc}	247.87 ± 8.45^{b}	245.56 ± 11.63^{bc}	236.78 ± 5.37^{bc}
	PM-2	278.94 ± 12.0^{a}	218.64 ± 7.59^{b}	235.61 ± 7.70^{b}	242.34 ± 15.31^{b}	242.64 ± 9.87^{b}	174.02 ± 4.88^{c}
Magnesium	PM-1	126.58 ± 6.37^{b}	142.72 ± 1.87^{a}	111.68 ± 2.29^{c}d	122.49 ± 3.08^{bc}	120.18 ± 5.32^{bc}	099.92 ± 5.42d
	PM-2	123.37 ± 5.62^{b}	138.92 ± 3.77^{a}	116.45 ± 5.27^{b}	118.03 ± 3.36^{b}	120.08 ± 5.07^{b}	099.35 ± 1.68^{c}

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters. PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165.

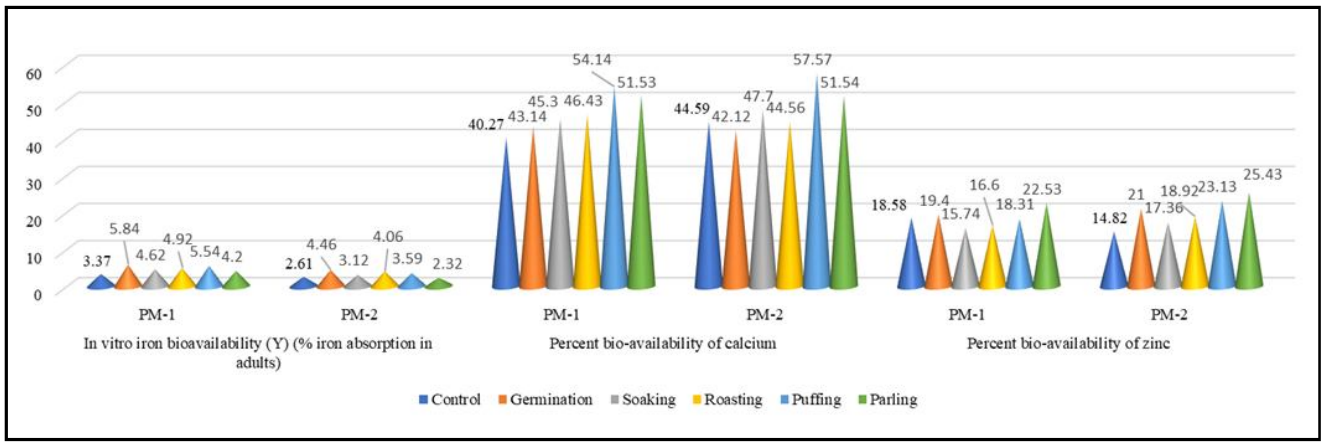

Figure 6: Effect of various treatments on *in vitro* bioavailability of minerals of pearl millet cultivars (dry matter basis).

4. Discussion

4.1 Nutritional composition

In the current study, germination was observed to enhance the protein and dietary fiber content of pearl millet cultivars. Similar results were reported by Akinola *et al.* (2017), displaying higher protein in malted pearl millet flour (15.39%). Such rise could be attributed to the utilization of carbohydrates and fats during respiration, resulting in loss of dry weight, withal microbial synthesis of some amino acids by germinating grains (Adebiyi *et al.,* 2017). The spike in dietary fiber content during germination could be due to structural disruption of polysaccharides in cell wall of the grain possibly affecting the anatomical intactness of tissue and hampering the carbohydrate-protein interaction leading to extensive biosynthesis of the new cell wall and producing new dietary fiber (Sharma *et al.,* 2015). However, the analyzed pearl millet cultivars in the current study also displayed an increase in dietary fiber content post-puffing which could be due to an increase in β-glucan availability accounting for the release of bound β-glucan due to the thermal effect (Kora, 2019). In addition, a reduction in the fat content of pearl millet flour post-germination was also recorded. A similar trend was reported by Jha *et al*. (2015) attributing it to hydrolysis of lipids and oxidation of fatty acids during grain germination.

4.2 Bioactive compounds

The effects of processing applications on the bioactive compounds of pearl millet flour in the current study revealed varied results. Correspondingly, Kadiri (2017) reported that some processing applications proved to improve the extractability, while others tended to degrade it. In the present investigation, germination was observed to enhance the total phenols (19.3-23.4%) content while other treatments tend to decrease it significantly. Evidence revealed that it could result in an action of cell wall-degrading enzyme, which became active due to germination, liberating bound phenolic molecules like hydroxycinnamates (*e.g*., ferulic and p-coumaric acids). The process of germination activates "esterases", which release bound phenols that are otherwise attached to non-starch polysaccharides in the grain's cell wall through ester and ether linkages (Duodo, 2014). Besides, the Phenylalanine ammonia lyase (PAL) enzyme involved in the manufacture of phytochemicals (phenols and flavonoids) is also stimulated by germination. The significance lies in the fact that this enzyme catalyses the metabolic trajectory of phytochemical biosynthesis while also regulating the rate of phenolic acid and flavonoid biosynthesis. Research studies, however, showed that germination increased the activity of this enzyme (Nkhata *et al.,* 2018). Another study by Devi *et al*. (2020) reported about a half of per cent reduction of total phenols post-popping makhana and attributed it to the thermal degradation of heat-labile phenolic compounds and dissociation of bran due to popping. Other investigators revealed that losses may result simply from leaching into the soaking water and may also be attributed to decreased extractability as lower molecular weight phenolic compounds polymerize, thus becoming insoluble in water or due to drifting of phenols from outer bran layers to endosperm during soaking, hampering its extractability due to binding of polyphenols with other organic substances such as carbohydrate or protein (Ramadan *et al*., 2012).

However, the tannin content of pearl millet flours analysed in the present investigation was observed to decline post-germination and soaking which could be credited to the leaching of tannins in the aqueous medium due to prolonged steeping. Additionally, the production and catalytic effect of enzymes (esterases) due to sprouting tend to hydrolyze the tannins, thus causing its reduction in the produced flour (Chethan *et al.,* 2008). In addition, popping caused the detachment of bran from the kernel, therefore, resulting in complete removal of tannins in popped makhana (Devi *et al.,* 2020). Likewise, the total flavonoid content of pearl millet variants reduced significantly post-processing except by germination. This observation could be associated with the removal of the bran layer during parling where flavonoids are concentrated while its heat-susceptible property might have reduced its content during roasting and puffing. Similar results were observed by Mir *et al*. (2016) who reported a significant loss of total flavonoid content post thermal application and informed a very high content in rice bran (178 mg/100 g), thereby owing the loss due to separation of bran during rice puffing.

4.3 Antioxidant activity

In both the pearl millet cultivars studied in the current study, a significant rise in the antioxidant activity by DPPH (12.4%) and FRAP (16.2-26.8%) due to germination was observed This surge could be attributed to the induction of high levels of enzymes (superoxide-dismutases, glutathione-S-transferase, peroxidises and catalases) with antioxidative properties (Gupta *et al.,* 2013). Also, an increase in the content of total phenols and flavonoids due to

germination contributed to enhanced antioxidant capacity as suggested by Owheruo *et al.* (2018) in their investigation. Research evidence reported that the existence of hydroxycinnamic and benzoic acid derivatives, as well as other flavonoids, is primarily responsible for the millet polyphenols' *in vitro* antioxidant ability. Additionally, esterases in the human gut have been found to cleave esterified hydroxycinnamates into free acids in the small intestine, and both ester-linked and free soluble phenolic acids may have some antioxidant effects on the luminal side of the intestinal tract. Besides, the presence of reductones (terminators of free radical chain reaction) had been reported to exert potent antioxidant activity of finger millet polyphenols (Banerjee *et al*., 2012).

However, a significant reduction in the antioxidant capacity (DPPH) after subjecting the millet flours to high temperatures (roasting and puffing) in the present investigation was also detected. This decline could be owed to the thermal degradation of heat-labile phytochemicals possessing antioxidant potential (Mir *et al*., 2016). Our observation corroborates with the results reported by Anjitha *et al*. (2021) in which the antioxidant activity of popped sorghum grain was observed to be inversely proportional to the rise in temperature from 200 to 300^{o}C. Nonetheless, parling proved to decrease the antioxidant activity of the millet flours studied in the current investigation. This could be owed to the removal of the bran layer in the process since, phenolics, flavonoids, and phytic acids were predominantly concentrated in the bran, which increased its antioxidant activity and caused diminution after the paring of the grains.

4.4 *In vitro* protein and starch digestibility

The present investigation recorded high protein digestibility of pearl millet flours specifically post-germination (61.6-70.5%) and thermal treatments (49.8-50.6%) which could be accredited to the release of a myriad of enzymes hydrolyzing biopolymers (storage proteins) making it easily available for pepsin hydrolysis and degrading anti-nutritional factors (Annor *et al.,* 2017). Thermal denaturation under apt conditions (roasting and puffing) increased the protein digestibility by unfolding the tight protein structure, increasing accessibility of hydrolytic enzymes to break long peptide chains by promoting racemization, Maillard reactions, formation of disulfide bridges and other covalent bonds such as lysinoalanine (LAL) and isopeptide bonds (Gilani *et al.,* 2012). An increase in starch digestibility by processing applications could be attributed to the production of hydrolytic enzymes during germination (α-amylase, β-amylase). Parling removes pericarp and germ, thus reducing anti-nutritional factors, fiber, protein, and lipids, leaving starch endosperm available for digestion. The enhanced starch digestibility by puffing and roasting could be because of intense starch gelatinization, thereby increasing the rate of starch hydrolysis (Huang *et al.,* 2018).

4.5 Minerals and their bioavailability

The present investigation recorded a reduction in iron content post germination (11.7-17.6%), soaking (10.6-15.6%), and parling (15.5-38.1%) which could probably be due to the transfer of nutrients to the developing embryo. Nazni and Devi (2016) reported a reduction of iron (16%) in germinated barnyard millet. However, Sihag *et al*. (2015) referred to the loss because of previous soaking and lixiviation of minerals. It is howbeit evident from recent studies and present investigation that parling results in high degree of mineral loss. Nanaiah *et al*. (2019) also reported that high iron content was directly proportional to loss by dehulling. However, an increase in the total calcium (17.8%) post-germination was observed while puffing (15.3%) and parling (34.3%) reduced the content. Chauhan (2018) also discovered a similar observation stating that oxalic acid decreased during sprouting which interferes with calcium absorption. The loss of mineral content post-parling and puffing in gun-puffed quinoa was owed loss of pericarp (Zapana *et al.,* 2020).

In the action of enzymes, zinc ions play both a structural and catalytic role. This antioxidant shields cells from the harm caused by oxygen radicals generated because of lymphocyte activation (Ifemeje *et al.,* 2015). The increase in the zinc content post soaking (18.5%) and germination (21.6%) of pearl millet flours was recorded in the current study which could be due to stimulation of esterase with hydrolysed tannins. However, parling caused a loss of zinc like reported loss (15.5-31.2%) post-dehulling by Nanaiah *et al.* (2019). Nonetheless, a decrease in the potassium post-processing pearl millet flours of both variants was observed. Results of the present investigation were comparable with Afify *et al.* (2012), reporting a decrease in potassium post-soaking (6.1-28.8%) and germinating (40.6-55.2%) sorghum cultivars. Analysis of phosphorus revealed a reduction in the content post-application of processing techniques. The results of the current study were in line with Chauhan (2018) which displayed a decrease (2.8%) of phosphorus by germinating finger millet.

Selective oxidoreductases require copper as a catalytic cofactor to function. Analysis of copper in the current investigation revealed an increase in the content post germination (3.8%) and soaking (1.4%) while roasting (8.3%), puffing (24.3%) and parling (49.6%) decreased the content. Results were comparable to those reported by Guardianelli *et al*. (2019), with an increase (7.5%) in the content post-germinating in amaranth grains whilst Thakur *et al.* (2021) reported an increase (26.2%) post-soaking and germinating of quinoa. Doddamani and Yenagi (2018) suggested that exposure to high temperatures (roasting) caused the loss of minerals in finger millet. However, parling caused loss of copper (33.2%) in PM-2 flour, while roasting increased (10.5%) the content. A similar finding was reported by Chauhan *et al.* (2022) who exhibited a slight increase in copper content (0.78-0.80 ppm) of roasted black soybean.

The magnesium content of pearl millet flours of the present investigation increased significantly post-germination. Observed results were in line with those reported by Owheruo *et al.* (2018) where an increase of 42 ppm in magnesium content of germinated pearl millet was reported. However, soaking and parling (20-21% approx.) reduced the content of both cultivars by approximately 6-12 and 20-21%, respectively. Similarly, Afify *et al.* (2012) reported a loss of magnesium after soaking three sorghum cultivars for 20 hours. Manganese content was found to be lower in puffed (10%), roasted (11.8%), and parled (50.4%) variants of PM-1 flour, whereas a very high notable loss of 57.8% was recorded for parled PM-2 flour. Nanaiah *et al*. (2019) reported a decline in manganese content by 12.3-26.2% after dehulling of sorghum. On the contrary, Guardianelli *et al*. (2019) and Thakur *et al*. (2021) reported an increase of 5.3 and 3.3% in the manganese content of germinated Amaranth.

In vitro bioavailability of calcium, iron, and zinc of the pearl millet flours studied in the present investigation increased significantly by processing with maximum absorption of iron by germination (68.5-71.3%), followed by puffing (64.4%). Abdalla *et al.* (2010) also reported an increase in mineral extractability of pearl millet by 23-70% post germination. This effect might be attributed to increased phytase activity, hydrolyzing phytate to phosphate and myoinositol phosphates, and boosting the availability of micronutrients (Chauhan, 2018). Amah *et al.* (2021) also demonstrated an increase (by 23-

26%) of iron content by roasting. An increase in ionizable iron and its bioavailability during soaking might be due to the breakdown of polyphenols-protein-minerals complexes and partial reduction of its phytate and polyphenol content (Sihag *et al.,* 2015).

Among the selected processing techniques puffed (53.5%) and parled (50.9%) variants of PM-1 displayed maximum calcium bioavailability. Johari (2017) also reported significantly higher calcium in germinated (31.47%) pearl millet flour. Zinc bioavailability also increased by parling (22.5-25.4%), germination (19.4-21%), and puffing (23.1%) pearl millet cultivars selected for the current study. In this regard, Amare *et al*. (2016) revealed that myo-inositol hexaphosphate (IP6- a mineral absorption inhibitor) content was reduced by 31 per cent resulting in an increase in mineral absorption.

5. Conclusion

Millets provide significant health benefits due to their high content of macro- and micronutrients, fiber, and phytochemicals, which can help combat chronic disorders. Making millet part of a regular diet can help to provide an affordable, sustainable, and healthy meal. However, the major constraint for the wide utilization of pearl millet is its low acceptability attributing to its undesirable flavor, which can be countered to some extent by applying processing techniques. It was observed that during the germination and puffing of two cultivars of pearl millet, nutrient retention, and bioaccessability improved. Simple processing techniques such as soaking, germination, and roasting can improve protein digestibility and the mineral's bioavailability, consequently helping to tackle the problem of malnutrition. Taking into consideration, the variability in the impact of processing on the nutritional profile of pearl millet, there is still a need to focus on optimizing the processing techniques for other millets to make them more acceptable without compromising the health benefits. In addition to this, to achieve the goal of sustainable food security, awareness needs to be created at both commercial and household levels regarding the impact of processing methods on the nutritional properties and health benefits of millet.

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Conflict of interest

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

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