

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2018; 7(6): 442-448 Received: 16-09-2018 Accepted: 18-10-2018

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Effect of hydrothermal treatments on mineral composition, bio accessibility and total polyphenols of pearl millet (*Pennisetum glaucum*)

Giri Snehal and Joshi Neena

Abstract

Millets are nutritionally superior to major cereals but contain anti-nutrients which are major phytochemicals which negatively affects their nutritive values. Millets requires specialized treatment in order to improve their nutritive value, shelf-life and organoleptic properties. For this hydrothermal treatments and coating treatments were employed in this study. Pearl millet grain was subjected to hydrothermal (microwave for 40, 60, 80 and 100sec and steamed for 4, 6, 8 and 10min) and curry leaf extracts (Ethanol, Hot water and Ethanol + water extracts) treatments. Untreated and treated grains were evaluated for mineral composition, iron and zinc bio-accessibility and for total polyphenol content. Increase in mineral content was observed in all treatments; polyphenols were significantly higher ($p \le 0.05$) in curry leaf extract treated grains than untreated grains; while steaming treatment showed more reduction compare to microwave treatment. Iron and zinc bio-accessibility was significantly ($p \le 0.05$) increased due to hydrothermal and curry leaf treatments. Treatments brought about an increase in accessibility of total minerals, iron and zinc and a decrease in the total phenolics, thus found to be an effective technique for improving the availability of minerals in pearl millet and making it nutritionally superior.

Keywords: Pearl millet, hydrothermal treatments, curry leaf extract treatment, total polyphenols, bio accessibility

Introduction

Pearl millet (Pennisetum glaucum) is family of gramineae originated in Africa and was subsequently introduced to India (Khairwal et al, 1990)^[16]. It is also known as Bajra. Pearl millet is the most widely grown type of millet. It accounts for about 50% of the total global production of millets. Pearl millet is well adapted to growing areas characterized by drought, low soil fertility, and high temperature. It is a coarse grain cultivated mostly in semi-arid parts of Africa and Asia (Shahidi and Chandrasekara, 2013) [32]. Millets are of great local importance as cheaper source of nutrients and staple for below poverty line population. Millet grains are nutritionally superior to major cereals with respect to protein, energy, vitamins, and minerals (Sehgal and Kawatra, 2003; Obilana and Manyasa, 2002) ^[3, 23]. Pearl millet contains an appreciable mineral content and exceptionally high iron content. Mineral content of pearl millet is 2.3 g/100g (Gopalan et al., 2012)^[10]. Pearl millet also contains anti-nutrients which are major phytochemicals includes tannins and phytates which form complexes with nutrients which negatively affect mineral absorption, palatability and protein digestibility by the human body. Processing reduces antinutritional content thereby increasing the mineral accessibility. (Pushparaj and Urooj, 2014)^[25]. Bioavailability or biological availability is term used to describe the proportion of a nutrient in food that can be utilized for normal body function (Hallberg et al., 1989) ^[12]. Bioavailability of minerals is an important function in food. Processing of grains normally has a positive impact through separation, partitioning or destroying inhibitors thereby enhancing their availability (Malleshi and Desikachar, 1985) ^[20]. Hydrothermal treatment is the process of subjecting a food to elevated temperatures in the presence of moisture, whereby the physicochemical properties of the starch within the food are modified, without destroying the granule structure (Adebowale et al., 2005)^[2]. It consists of soaking, steaming and drying of the food grains. However, information of these treatments is limited to few types of millet. This process is commonly applied to rice worldwide (Bhattacharya and Ali, 1985)^[5] and to wheat to some extent (Bayram, 2000)^[4]. Parboiling of sorghum and pearl millet has also been explored by Serna-Saldivar and Rooney (1995) [31]. Several studies also have shown that such treatments may reduce or increase the phenolic content depending on severity of treatment, time of exposure and type of grain tested (Hegde

and Chandra, 2005) ^[13]. The edible coatings are also used for shelf life extension and to improve nutritive value of food products (Falguera et al., 2011)^[9]. Several antioxidant rich ingredients have been incorporated in cereal foods to improve their shelf life (Ranjitha, 2013) ^[29]. Herbal treatments were tried on different food systems for shelf life extension. Among different treatments such as aloevera, fenugreek seed paste, gum acacia and curry leaves, Wahlang (2014) [39] observed that curry leaf paste treatment had the greatest impact on retardation of rancidity. Curry leaf (Murraya koenigii Spreng), is an aromatic small tree belonging to citrus family, Rutaceae, that most often grows in East Asia. Curry leaf is very well known for its high polyphenol content and antioxidant activity. The anti-oxidant activity of the curry leaf is due to the presence of carbazole alkaloids-mahanimbine, murrayanol, murrayanine, mukonine, murrayafoline-A, girinimbine, euchrestine B, mahanimbine and mahanine; koenigine; glycoside; triterpenoids and polyphenolic compounds (Devatkal et al., 2012; Tembhurne and Sakarkar, 2010) [8, 37]

In this direction, the purpose of the present study was to determine the impact of hydrothermal treatments such as steaming and microwaving and seed treatment a comparative evaluation of curry leaf extract coating treatment was performed with different extracts of curry leaves on minerals, mineral bio-accessibility and total polyphenols on pearl millet.

2. Materials and Methods

2.1. Materials

Pearl millet grains (10 kg) grown in the kharif season of year 2015 were procured from one farmer from Chitradurga, Karnataka. The grains were thoroughly cleaned by sieving for removal of sand particles, dust, foreign materials and broken seeds and were used for further studies. Grains were packed in a polyethylene bag and stored in a refrigerator at 18⁰ in freezer. Standard phenolic compounds, gastric digest, pepsin, pancreatin and bile extract of porcine origin were procured from Sigma–Aldrich Chemical Co. All other chemicals and reagents used in the experiments were of analytical grade.

2.2. Preparation of grain treatments

Pearl millet grains were subjected to hydrothermal treatment and curry leaf extract coating treatment before milling into flour. Initially moisture content of pearl millet grain was analyzed. Untreated pearl millet was taken as control.

2.2.1. Hydrothermal treatments of pearl millet a. Microwave treatment of pearl millet

The grains were placed in small cylindrical drum, moisture content of grains were adjusted to 18% by adding distilled water and tumbling of grains was done for 10 minutes. Hydration to 18% moisture content was achieved by the procedure outlined by Yadav *et al.* (2012) ^[40]. After tumbling, the moistened grains were kept in beaker for 1 hour and shaken occasionally for even moisture distribution. These grains were treated in microwave oven for 40, 60, 80, 100 sec at 900W. Grains were removed from microwave and temperature was measured. Grains were dried at ambient condition.

b. Steaming treatment of pearl millet

For steaming treatment, the procedure described by Mannuramath and Yenagi, $(2015)^{[22]}$ was followed. Grains were soaked in hot water (65°C) for 2 hours. The water was

removed and grains were drained and tied in muslin cloth and were steamed in pressure cooker for 4, 6, 8, 10 min. Grains were dried under shade at room temperature.

2.2.2. Curry leaf treatment of pearl millet a. Curry leaf extract preparation (Ref)

Fresh curry leaves were washed and dried. The leaves were ground using a grinder. The powder was stored in freezer until use.

For pearl millet, coating three extracts, namely: 80% ethanol extract of curry leaf, hot water extract and ethyl alcohol: water extract of curry leaf were used for coating. Solvent extract of homogenized ground curry leaf powder was prepared by weighing 5 gram of curry leaf powder in 20 ml of 80% ethanol, hot water and ethyl alcohol: hot water (1:1) respectively. The mixture was kept for shaking in water bath shaker for 30 min and centrifuged for 15 min at 5000rpm. Supernatant was filtered using Whatman filter paper No. 1. The procedure was repeated for three times to ensure complete extraction. All the three supernatant were pooled and evaporated till dryness.

b. Preparation for curry leaf extracts coating

All three extracts from 5 g of curry leaf namely EEC, HWC and EWC were mixed well with 2.5% gum acacia and used 20 ml water per 100 g pearl millet separately and used as a coating respectively.

2.3. Chemical analysis

The dried grains were ground into flour. Flour was then packed in HDPE pouches, sealed with sealer and stored in freezer (-18 °C) until use. The following nutrients namely ash, manganese, iron, zinc, copper, sodium and total phenolic content were also analyzed. Zinc and iron bio-accessibility were analyzed by standard procedures described hereunder. All samples were analyzed in triplicates.

2.3.1. Estimation of total ash

The total ash content was determined as per the procedures outlined by Association of Official Analytical Chemists (AOAC, 1980) ^[1]. Total ash was estimated in the sample by weighing about 5g of dried sample into a weighed crucible (W_1). The crucible was placed on a wire gauze and heated over a low flame till the material was completely charred and fumes ceased to appear. Crucible was then ignited in a muffle furnace for 5-6 hours at 550±25°C. It was cooled in desiccator and weight of ash was observed. It was then cooled for one hour and weighed. This was repeatedly done till two consecutive weights were the same and the ash was almost white or grayish colour.

Total ash (g /100 g) =
$$\left(\frac{\text{initial weight of sample-final weight of ashed sample}}{\text{weight of sample(g)}}\right) \times 100$$

2.3.2. Estimation of minerals

To the ash 5 ml of a 1:1 solution of distilled water and fuming HCl was added. Minerals were estimated using prepared mineral solution. Zinc and iron content of the sample was estimated by using atomic absorption spectrophotometer and the result was expressed as mg/100 g of the samples as described by Page *et al.* (1992) ^[24]. The copper and manganese content was estimated using atomic absorption spectrophotometer by using Dithizone method (AOAC, 1980) ^[1] and sodium (Na) content in the extracts was estimated by

digital flame photometer after calibrating with standard sodium solutions (Ranganna, 2000)^[27].

2.3.3. Determination of bio accessibility of iron and zinc

in vitro digestion was done using the method of Luten *et al.*, 1996. Bio accessibility of iron was assessed using the method of Rao and Prabhavati (1978) ^[28] and bio-accessibility of zinc was also done with slight modification of the same method. **Gastric digestion**: 10 g of sample was mixed with 80 ml of distilled water in a beaker. pH was adjusted at 2.0 with 6M HCl. pH was rechecked after 15 min and adjusted to 2.0. Three ml of pepsin was added to pH adjusted sample then made up the volume to 100 ml. The mixture was incubated at 37 °C for 2 hrs shaking and then stored at 0°C for 90 min. After keeping for 90 min titrable acidity was estimated

Titrable acidity: 20 ml of gastric digest was mixed with 5ml of pancreatin mix. pH of gastric digest was adjusted to 7.5 with 0.2 M NaOH, rechecked after 30 min and adjusted to 7.5. Finally the quantity of NaOH required to neutralize with NaHCO₃ was equated.

Membrane processing: Membrane was cut around 9" length and it was boiled in distilled water in a beaker for 3 to 4 times. One side of the membrane was tightly tied with thread and calculated NaHCO₃ was poured in the bag and membrane was tied the other side.

Intestinal digestion: 20 ml gastric digest was equilibrated to 37 °C for 5 min in water bath. Dialysis tube with needed quantity of NaHCO₃ was taken. The content was incubated at 37° C till the pH reached to 5. Five ml of pancreatin mix was added once pH reaches to 5 since pancreatin works at lower pH. Incubation was continued in the shaker till the pH reaches to 7.0 (2 hrs or whole night). Dialysis tube was removed from the shaker, rinsed with water. Content was drained out and the iron content was estimated.

2.3.4. Determination of total polyphenols

Folin-Ciocalteau method was used for estimation of total phenolic content (Singleton et al., 1999) [34]. Before estimation of total polyphenol content, the extraction of ground pearl millet was done. For this 2g of sample was extracted in a centrifuge by using 80% methanol maintained at pH2 by help of 6N HCl and centrifuged at 5000 rpm for 15 min. The process of extraction was repeated three times. The extract was stored in air tight container (-10 °C). This extract was used for estimating total phenolic content. The mixture was prepared by mixing 0.1 ml of methanolic solution of extract and volume made up to 1.5 ml by distilled water, in this 0.5 ml of 50% Folin-Ciocalteu's reagent and 10 ml of 7.5% NaHCO₃ was added. Blank was concomitantly prepared, containing 0.1 ml methanol, and volume made up to 1.5 ml by distilled water. To this 0.5 ml 50% Folin-Ciocalteu's and 10 ml of 7.5% of NaHCO₃ was added. The samples were thereafter incubated in a thermostat at 45° C for min. The absorbance was determined 45 using spectrophotometer at λ max = 765 nm. The same procedure was repeated for the standard solution of gallic acid.

Calculation of total polyphenolic compound (TPC):

TPC (mg GAE/100 gm) = $\frac{\text{Std.Conc.}}{\text{Std 0.D}} \times \frac{\text{Sample 0.D.}}{\text{Aliquot taken}} \times \frac{\text{Volume make up}}{\text{weight of sample}} \times \frac{100}{1000}$

2.6. Statistical analysis

Data was in triplicates and these values were used for statistical analysis. Analysis of variance (F-test) was done by using ICARGOA for minerals, bio accessibility and antinutritional factors to test the significant difference between samples in the study. Difference were declared statistically significant when $P \le 0.05$.

3. Results and Discussion

3.1. Effect of hydrothermal and coating treatments on total minerals

Minerals are essential nutrients for human well-being and they play a vital role in the effective functioning of the body activity (Wang *et al.*, 2011). Minerals have various functions in the body. About 4-5 percent of the human body weight is made up of mineral content, 4/5 of this being in the skeleton and the rest in other organs (Manay and Sadaksharswamy, 2005) ^[25]. Pearl millet is nutritionally superior to most other cereals, having high levels of calcium, iron, zinc, lipids and high quality proteins (Klopfenstein and Hoseney, 1995). Therefore, it becomes essential to assess the mineral content of any food product and effect of various treatments on it. Various types of processing have different effects on mineral composition of a product. Therefore, various micro and macro minerals were evaluated in the study.

A significant difference was found in ash content of hydrothermally treated and untreated pearl millet. It ranged from 1.55 to 2.08 g/100g. The values are slightly lower than those reported by Gopalan *et al.* (2012) ^[10], which is 2.3 g/100 g. Untreated grains (6.49 mg/100 g) have shown lowest iron content and curry leaf aqueous extract grains had the slightly higher amount of iron (9.49 mg/100 g) than other two treatments. It can be because of addition of curry leaf iron content. There was no significant differences existed between all treatments. The iron values in the raw, conventional cooked and solar cooked pearl millet was reported as 10.38, 9.93 and 10.20 g/100 g respectively (Pushparaj and Urooj, 2014) ^[25].

Zinc content of treated and untreated pearl millet ranged from 3.09 to 3.58 mg/100 g. The maximum (3.58 mg) and minimum zinc content (3.09 mg) was observed in pearl millet grains pretreated with steam for 6 min and microwave treated for 60 sec respectively. Values were lower than those reported by Tripathi *et al.* (2010) ^[38] on pearl millet flour, (4.04 mg/100 g). Variation in the content was because of varietal difference.

In this study copper and manganese content of untreated and treated pearl millet was ranged from 0.21 to 0.32g/100g and 0.60 to 0.76 mg/100 g respectively. These values were slightly lower than the values reported by Gopalan *et al.* (2012) ^[10] for copper (1.06 mg/100g) and manganese (1.15 mg/100 g). The reason can be the varietal difference in the pearl millet grains. There was a significant difference in the manganese content between treated and untreated samples.

The sodium content of the treated and untreated pearl millet ranged from 6.27 to 11.87 mg/100 g. Untreated sample had the highest sodium (11.87 g/100 g) and treated grains had considerably low sodium whereas lowest was found in microwave treated grains for 80 sec (6.27 mg/100 g). Lower values in hydrothermally treated pearl millet may be due to the leaching of sodium while soaking. Leder (2004) ^[17] reported 10.9 mg of sodium in samples of untreated pearl millet which are closer to values in this study.

Hydrothermal processing did not cause significant changes in the nutrient contents of the millet. A slight decrease in the content of some of the nutrients could be due to the loss during steeping.

3.2. Effect of treatments on total polyphenol content

The food contains a wide range of organic chemical compounds along with different nutrients. Some of these compounds have an anti-nutritional function which interferes with the utilization of some of the nutrients such as protein, calcium iron phosphorous etc present in the foods particularly those of plant origin. The main anti-nutritional factors are phytates, trypsin inhibitors, certain phenolic compounds and dietary fiber (Camara and Amaro, 2003) ^[6]. Therefore, it is important to assess the polyphenol content in millet samples. All plant-based foods have phenols, which affect their appearance, taste, odor and oxidative stability. In grains, these phenolic acids are located in the pericarp, testa, aleurone layer, and endosperm (Hahn *et al.*, 1984) ^[11]. Pearl millet contains many anti-nutritional factors which may limit the absorption of essential minerals. The total polyphenol content was expressed as mg Gallic acid equivalent per 100 g sample (mg GAE/100 g) is shown in Tables 2. The total polyphenol content (TPC) ranged from 239.2 to 148 mg GA/100 g. Untreated grains had 234.4 mg GA/100 g polyphenol content and is lower than one reported by Archana and Kawatra (1998) (764.45 mg/100 g) and slightly higher than that reported by Ragaee et al. (2006) ^[26] (138 mg GA/100 g). The differences in the values can be easily attributed to the varietal differences. Polyphenol content of microwave and steam treated grains ranged from 175.5 to 191.0 mg GA/100 g and 148.0 to 170.2 mg GA/100 g respectively. Among all treatments, the highest amount of total polyphenol content was found in curry leaf ethanol extract treated grains (239.2 mg GA/100 g) and the lowest in the grains pretreated for 10 min steaming (148.0 mg GA/100 g). Ethanol extract of curry leaf had higher amount of total polyphenol followed by ethanol+aqueous (1:1) and lowest in aqueous extract was reported by Singh et al. (2011) ^[33]. There was decrease in total polyphenol content in steamed treated grains compared to microwave treatment. The reason could be due to the reduction in polyphenol content after treatment. Hydrothermal treatment reduces polyphenol content of the grain due to oxidative and degradation reactions, take place due to exposure to heat (Chandrasekara et al., 2012) ^[7]. Simple processing techniques like dehulling and decortications decrease polyphenol and phytic acid content of pearl millet (Hegde and Chandra, 2005) ^[13]. The graphs of total polyphenol content of pearl millet with various treatments are shown in fig. 1, 2, 3 and 4.

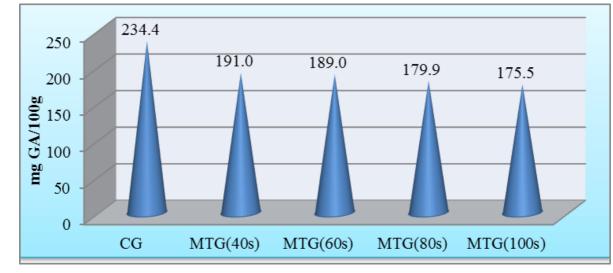


Fig 1: Total polyphenol content (mg GA/100g) of pearl millet flour subjected to hydrothermal microwave treatment. CG- Control untreated; MTG (40s), MTG (60), MTG (80s) and MTG (100s)- Microwave treated for 40, 60, 80, 100sec respectively.

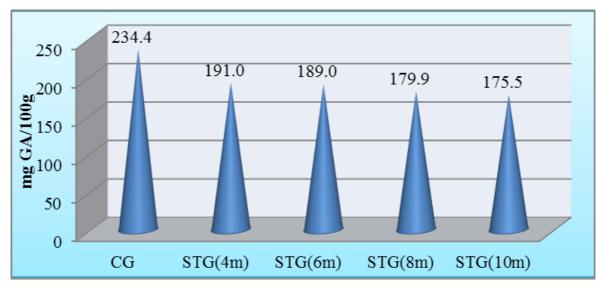


Fig 2: Total polyphenol content (mg GA/100g) of pearl millet flour subjected to hydrothermal steaming treatment. CG- Control untreated; STG (4min), STG (6min), STG (8min) and STG (10min)- Steamed for 4, 6, 8, 10min respectively.

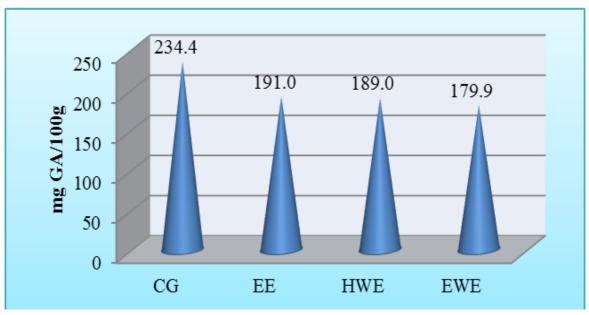


Fig 3: Total polyphenol content (mg GA/100g) of pearl millet flourpretreated with various extracts of curry leaf. CG- Control untreated; EEethanol extract, HWE- Aqueous extract, EWA- Aqueous + ethanol extract.

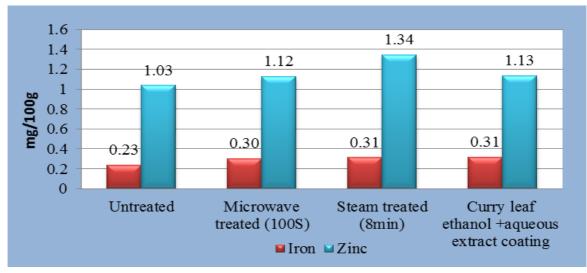


Fig 4: Bio-accessible iron and zinc (mg/100g) in pearl millet with different treatments.

Treatments	Ash (g/100 g)	Fe	Zn	Cu	Mn	Na
		(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)
	-	Control				
Untreated	1.94	6.49	3.20	0.26	0.72	11.87
	Hydrothermal	microwave trea	tment (900W)			
40 sec	1.90	7.15	3.09	0.32	0.67	6.87
60 sec	2.08	7.25	3.09	0.25	0.65	6.53
80 sec	2.00	7.05	3.12	0.25	0.60	6.27
100 sec	1.92	7.20	3.27	0.21	0.67	6.73
	Hydroth	nermal steam ti	eatment			
4 min	1.55	7.04	3.22	0.21	0.61	8.47
6 min	1.60	7.19	3.58	0.23	0.73	8.67
8 min	1.57	7.15	3.43	0.25	0.64	8.67
10 min	1.55	7.13	3.29	0.27	0.60	7.53
	Curry	leaf extract tre	atment			
Ethanol extract	2.01	9.09	3.55	0.31	0.76	7.27
Aqueous extract	2.10	9.49	3.25	0.27	0.72	8.80
Ethanol + aqueous extract (1:1)	2.05	8.84	3.13	0.29	0.74	7.33
F value	128.9*	13.13	2.35*	2.91*	4.76*	13.52*
SEm ±	0.02	0.26	0.11	0.02	0.02	0.40
CD (P≤0.05)	0.057	NS	0.322	0.063	0.074	1.16

*Significant at 5% level, NS Non-significant

Table 2: Total polyphenol content (mg GA/100 g) of pearl millet
flour from grains subjected to different treatments.

Treatments	TPC (mg GA/100 g)				
Control					
Untreated	234.4				
Hydrothermal microwave (reatment (900W)				
40 sec	191.0				
60 sec	189.0				
80 sec	179.9				
100 sec	175.5				
Hydrothermal steam	treatment				
4 min	170.2				
6 min	164.4				
8 min	156.2				
10 min	148.0				
Curry leaf extract t	reatment				
Ethanol extract	239.2				
Aqueous extract	236.8				
Ethanol + aqueous extract (1:1)	229.1				
F value	67.75*				
SEm ±	4.060				
CD (P≤0.05)	11.85				

* Significant at 5% level

3.2. Iron and zinc bio accessibility

Pearl millet tough fibrous seed coat is rich in antinutrient like phytic acid, tannins and oxalates that forms complexes with dietary minerals such as zinc, iron and makes them biologically unavailable (Pushparaj and Urooj, 2011). Iron and zinc are an essential micronutrient of human body. Nowadays, the low bio availability of iron and zinc has become a serious health issue. Thus, it becomes important to assess the bio accessibility of iron and zinc. Bio accessibility is referred to the amount of compound that is released from the solid food matrix into the gut while bio availability is defined as an amount of nutrient in a food that is absorbable and utilizable by the person consuming the food in a typical meal (Lestienne *et al.* 2005) ^[19].

The in vitro bio-accessibility of iron and zinc in pearl millet grains was done by imitating human gastrointestinal system (Table 3). Bio-accessible iron and zinc was estimated for untreated grains (control) and one treatment each of the two types of hydrothermal treatments that is steaming and microwaving. Untreated pearl millet showed lowest iron and zinc bio-accessibility that is 0.23 and 1.03 mg/100g respectively. This could probably be attributed to the higher amounts of inhibitory factors such as phytate and tannin present in pearl millet flour. Grains steamed for 8 min showed highest iron and zinc bio accessibility 0.31 and 1.34 mg/100g respectively (Fig. 4). Improvement in bio accessible iron and zinc in steaming treatment could be attributed to the decrease in polyphenol content; solubilization of insoluble fiber also heating treatment softened the food matrix and thereby increased absorption of these minerals. Soaking and boiling processes have been shown to reduce the phytate content by Sushma et al., (2008) [36]. Grains treated with microwave for 80sec have shown slightly lower values compare to steamed grains. The values reported by others for iron and zinc were 0.10 g/100 g, 0.12 g/100 g and 0.94 g/100 g, 0.64 g/100 g for untreated endosperm and water soaked endosperm of pearl millet respectively (Jha et al., 2015) ^[15]. Simple processing techniques like soaking, germination and cooking were reported to have reduced anti-nutrient content and increased the bioavailability of iron and zinc (Suma and Urooj, 2011) ^[35]. Bio-accessibility of zinc was significantly higher when compared to iron thus, an increase demand for iron consumption occurs. The various treatments in this study have increased the iron content of the grain which can be used as a vehicle for increased iron content.

 Table 3: Bio-accessible Iron and Zinc (mg/100 g) of pearl millet flour treated with various treatments.

Treatments		Zinc
Untreated		1.03
Hydrothermal Microwave treated (100 sec)		1.12
Hydrothermal steam (8 min)		1.34
Ethanol +aqueous curry leaf extract		1.13
F value		26.82*
SEm±		0.01
CD (P≤0.05)	0.05	0.09

4. Conclusion

Pearl millet (Pennisetum glaucum) is the most widely grown type of millet and it is staple diet for farm households in the world's poorest countries and among the poorest people. Present study revealed the hydrothermal treatment and curry leaf extract coating treatment of pearl millet brought the reduction of total polyphenol content without affecting the mineral composition. Bioaccessibility of iron and zinc was more after giving treatments. Therefore, from the present investigation, it could be established that one of the major constraints in widespread utilization of pearl millet could be successfully and appreciably avoided by using these simple processing treatments. Thus, hydrothermal treatment is an effective process to reduce anti-nutrients without affecting the mineral composition and to improve mineral bio-accessibility. The use of hydrothermal processing technique, if adopted, could be instrumental in raising the acceptability and nutrient availability of pearl millet products.

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