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Cogent Food & Agriculture (2015), 1: 1109171



Received: 02 September 2015
Accepted: 01 October 2015
Published: 29 October 2015

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Reviewing editor:
Fatih Yildiz, Middle East Technical University, Turkey

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Effect of domestic processing treatments on iron, β -carotene, phytic acid and polyphenols of pearl millet

Manvesh Kumar Sihag^{1*}, Vivek Sharma², Ankit Goyal¹, Sumit Arora² and Ashish Kumar Singh³

Abstract: The objective of the present study was to evaluate the effect of various processing treatments (individual and combination) on iron, β -carotene, phytic acid, polyphenols and ash content of pearl millet (*Pennisetum americanum*). Grains were subjected to soaking, pressure cooking, steaming, malting, pearling and extrusion cooking for different time intervals such as soaking for 3, 6, 9 and 12 h; steaming for 5, 10, 15 and 20 min; pressure cooking for 2, 5, 7 and 10 min; controlled germination (malting) for 12, 18, 24, 36, 40, 46 and 52 h along with three combinations of treatments. Data revealed that phytic acid was reduced maximum (38.23%) by malting, whereas polyphenols (49.28%) and ash content (22.09%) were decreased maximum by pressure cooking. Loss of β -carotene and iron was also higher (29.79, and 16.03%, respectively) during pressure cooking in comparison to other processing methods. However, combined treatments showed higher retention of β -carotene and iron with more reduction of anti-nutrients over the individual treatments. Overall, it can be concluded that combination of domestic treatments is a better approach in improving the nutritional profile of pearl millet, which can be consumed directly or as one of the ingredients for formulations like weaning foods, bakery products, etc.



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PUBLIC INTEREST STATEMENT

Several crops like pearl millet, sorghum and maize can grow under adverse agro-climatic conditions such as little irrigation facilities and infertile land. These crops are also termed as “nutri-cereals” and could be a potential source of micronutrients such as iron and zinc. If correct combination of various processing treatments (soaking, germination, pressure cooking and steaming) is employed to reduce the level of anti-nutrients such as phytic acid and polyphenols, their nutritional attributes could be improved with better bioavailability. Foods prepared by these domestically processed underutilized cereals could be an effective replacement of high cost processed food products available in the market with similar nutritional profile; and could be a better approach to deal with the malnutrition and micronutrients based deficiency diseases such as anemia in developing and underdeveloped countries worldwide.

Subjects: Food Analysis; Nutraceuticals & Functional Foods; Nutrition

Keywords: β -carotene; malting; pearl millet; phytic acid; polyphenols; pressure cooking

1. Introduction

Pearl millet (*Pennisetum americanum*), also known as Bajra in India, is an important food crop of South Asia and Africa. Because of its sustainability under adverse agro-climatic conditions, it is also termed as crop of food security. India is the largest producer of pearl millet both in terms of area and production (Yadav, 2014). It is also termed as “nutricereal” due to presence of complex carbohydrates (67.5%), high proportion of dietary fibers, and other phytochemicals with nutraceutical properties (Sumathi, Ushakumari, & Malleshi, 2007). The protein’s biological value and digestibility coefficient of pearl millet were measured as 83 and 89%, respectively. The protein efficiency ratio of pearl millet (1.43) was more than that of wheat (1.2) (National Research Council, 1996).

Pearl millet is known for its high amount of macro- and micronutrients (such as B-vitamins, potassium, phosphorous, magnesium, iron, zinc, copper, and manganese) (Sihag et al., 2015). However, it contains significant amounts of anti-nutrients also, such as polyphenols, enzyme inhibitors, and phytates. Antinutrients are associated with the low bioavailability of minerals and proteins. Humans and other non-ruminant animals cannot digest phytates due to the absence of digestive enzyme phytase. It is usually found as a complex with essential minerals and/or proteins. The actual mechanism of the interactions between phytic acid and minerals are yet to be understood, although it is possible that it could form a complex with a cation on the same or different molecules within a simple phosphate group or between two phosphate groups (Hithamani & Srinivasan, 2014). Similarly, polyphenols also act as anti-nutrients and chelates divalent metal ions like iron and zinc and reduce their bioavailability. They also inhibit digestive enzymes and may also precipitate proteins. Various processing treatments have been reported which can reduce the level of anti-nutrients, such as soaking, germination, steaming, fermentation, microwave heating, etc. Several researchers have studied the effect of various processing treatments on the content of anti-nutrients of different cereals/legumes (Goyal & Siddiqui, 2014; Goyal, Siddiqui, Upadhyay, & Soni, 2014; Osman & Gassem, 2013; Rao & Muralikrishna, 2001). Sharma, Goyal, and Barwal (2013) studied the effect of soaking and cooking on polyphenols, tannins, and phytates and reported approximately 14.7–45.1% reduction in soybeans. Similarly, Hithamani and Srinivasan (2014) investigated the effect of domestic processing on the polyphenol content in pearl millet (*Pennisetum glaucum*) and observed that sprouting and pressure cooking reduced 33.52 and 41.66% polyphenols, respectively. It is clear from the literature discussed above that several workers have worked on this aspect, but the effect of combination of various domestic treatments on reduction of anti-nutrients along with the impact on the loss of β -carotene and iron is hardly discussed in pearl millets.

In this context, the objective of the study was to optimize the process to develop pearl millet flour with minimum loss of iron and β -carotene and maximum reduction of anti-nutrients, so that it could serve as a base component for the formulation of pearl millet-based weaning foods.

2. Materials and methods

2.1. Materials

Pearl millet grains (Pro-Agro’s 9444) were procured from pearl millet breeding farm, Haryana Agriculture University, Hisar (India). Airtight plastic containers were procured for grain and flour storage. All solvents and reagents used in this study were of analytical grade and were purchased from Himedia, India and Merck, Germany.

2.2. Different combinations of treatments for pearl millet processing

The pearl millet grains were subjected to four different processing treatments, viz. soaking, pressure cooking, steaming, and controlled germination (malting) at different time intervals (Table 1).

Table 1. Optimization of parameters for pearl millet

Treatment	Time
Soaking at 30°C	3, 6, 9 and 12 h
Pressure cooking	2, 5, 7 and 10 min
Steaming	5, 10, 15 and 20 min
Controlled germination at 25°C and 80% humidity	12, 18, 24, 36, 40, 46 and 52 h
Soaking + germination + pressure cooking	9 h + 40 h + 5 min
Soaking + germination + pearling	9 h + 40 h + pearling
Soaking + germination + pearling + extrusion	9 h + 40 h + pearling + extrusion at 110°C

2.2.1. Soaking/steeping

The raw, clean grains were soaked in water in the ratio of 1:4 for 3, 6, 9, and 12 h at $25 \pm 2^\circ\text{C}$. The soaked grains were stirred periodically in order to remove the gases accumulated around the grains and the steep water was changed after every 2 h interval to prevent the growth of undesirable microbes. The grains were dried at $60 \pm 2^\circ\text{C}$ using tray dryer to 13% moisture content.

2.2.2. Steaming

The raw, clean grains were steamed in a cooker (by detaching the whistle from the lid of the pressure cooker) for 5, 10, 15 and 20 min. The ratio of grains to water was kept 1:4. The steamed grains were then dried at $60 \pm 2^\circ\text{C}$ using tray dryer to $13 \pm 0.50\%$ moisture content (dry weight basis).

2.2.3. Pressure cooking

The raw, clean grains were pressure cooked (the whistle was attached with the lid) in a cooker for 2, 5, 7 and 10 min. The ratio of grains to water was kept 1:4. The grains were then dried at $60 \pm 2^\circ\text{C}$ using tray dryer to $13 \pm 0.50\%$ moisture content.

2.2.4. Controlled germination

Controlled germination of pearl millet grains was carried out for different time intervals, viz. (12, 18, 24, 36, 40, 46 and 52 h) at $25 \pm 2^\circ\text{C}$. The washed grains were allowed to germinate between the folds of muslin cloth in an incubator ($25 \pm 2^\circ\text{C}$). Water was sprinkled intermittently to moisten the muslin cloth. After malting, the grains were dried at $60 \pm 2^\circ\text{C}$ using a tray dryer till the moisture content reached about $13 \pm 0.50\%$. The rootlets of germinated and dried grains were removed by scrubbing manually over a perforated tray.

2.2.5. Pearling

The raw and germinated pearl millet grains were pearled in a pearling machine to remove the outer greyish layers of the grains. Pearling was done by rubbing the grains against the abrasive stones and air pressure was used to remove the loosened bran layers. The degree of removal was regulated by controlling the time of pearling and by adjusting the space between the abrasive stones and the screen. The grains were weighed before and after the pearling in order to calculate the degree of pearling. Pearling was done for 40 s corresponding 15–20% removal of the husk. The grains were cleaned to remove the fine pearling with the help of lab aspirator. Pearled grains were subjected to extrusion processing described as follows.

2.2.6. Extrusion processing

Prior to extrusion processing, grains were preconditioned to adjust the feed moisture content. The calculated amount of water was sprinkled over the pearl millet grains to increase the feed moisture content to $13 \pm 0.5\%$. The moistened grains were kept for 48 h for preconditioning in airtight polythene bags to equilibrate moisture. After 48 h of conditioning, grains were fed into feeder hopper that contained screw auger to transport materials at uniform rate into the barrel. The single screw extruder consists of one screw in the barrel to transport the ingredients through its three zones, viz.

feeding zone, kneading zone, and cooking zone. The temperature of cooking zone was maintained at 110°C. The material was finally extruded through a 3 mm diameter die where it expanded due to sudden evaporation of water from plasticized mass. It was cut into pieces of desired size by a cutter which was adjusted at 570 rotations per min (RPM) with feeder speed of 115 RPM and motor speed 1,300 RPM. Finally, extrudates were milled in the milling machine to obtain the flour with a fine sieve attached. The flour was kept in airtight plastic containers for further use.

2.3. Milling

The moisture content of pearl millet grains was maintained to 13% by conditioning. The conditioned grains were milled in roller mill (Chopin Laboratory CD-1 mill, France).

2.4. Total polyphenols content estimation

Total phenolic content was determined by Folin–Ciocalteu spectrophotometric method (Gao, Wang, Oomah, & Mazza, 2002).

2.5. Phytic acid

Phytic acid content was determined using Megazyme assay kit (Wicklow, Ireland).

2.6. Ash and iron content

Ash and iron content were estimated by the standard methods of Association of Analytical Chemists (2005). Iron was estimated using Atomic Absorption Spectrophotometer (AAS) using dry digestion.

2.7. β -carotene estimation

β -carotene extraction, saponification was performed by the method of Howe and Tanumihardjo (2006), while estimation in all the treatments and extruded pearl millet was carried out using method of Sanusi and Adebiji (2009).

2.8. Statistical analyses

Means ($n = 3$), standard error mean (SEM), linear regression analysis, and 95% confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data were subjected to a single-way analysis of variance (ANOVA) to calculate CD value.

3. Results and discussion

3.1. Effect of soaking

Phytic acid and total phenol contents of raw pearl millet flour were found to be 683.07 ± 1.87 and 207.23 ± 3.06 mg per 100 g, respectively (Table 2). Phytic acid content decreased significantly ($p < 0.05$) as the soaking time increased. Similarly, total polyphenol content was also reduced significantly ($p < 0.05$) from its initial value of 207.23 to 198.96 mg and 183.49 mg after 9 and 12 h of soaking, respectively. β -carotene level was not affected significantly up to 9 h of soaking, however,

Table 2. Effect of soaking on anti-nutrients, β -carotene, iron and ash content of pearl millet

Time (h)	Phytic acid (mg/100 g)	Total polyphenols (mg/100 g)	β -carotene (μ g/100 g)	Iron (ppm)	Ash (%)
0	683.07 ± 1.87^a	207.23 ± 3.06^a	85.74 ± 0.82^a	43.23 ± 0.28^a	1.72 ± 0.007^a
3	674.43 ± 2.08^b	206.16 ± 3.22^{ab}	84.88 ± 0.53^{ab}	42.35 ± 0.12^b	1.68 ± 0.008^b
6	648.17 ± 1.45^c	201.89 ± 3.79^{ab}	83.86 ± 1.22^{ab}	40.3 ± 0.19^c	1.63 ± 0.004^c
9	624.44 ± 1.71^d	198.96 ± 2.55^b	83.76 ± 1.41^{ab}	39.37 ± 0.34^d	1.59 ± 0.002^d
12	616.72 ± 1.59^e	183.49 ± 1.99^c	82.63 ± 0.99^b	38.51 ± 0.46^e	1.55 ± 0.002^e

Notes: Data are presented as means \pm SEM ($n = 3$), Means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

after 12 h of soaking, it was reduced significantly ($p < 0.05$). Iron and ash content reduced significantly ($p < 0.05$) as the soaking time was increased. Our findings are in accordance with findings of other workers who observed decrease in phytic acid content of cereal grains meant for the production of weaning foods by soaking and germination (Gupta & Sehgal, 1991; Osman & Gasseem, 2013). Similar reduction in phytic acid concentration during soaking in pearl millet was also reported by other workers (Duhan, Chauhan, Punia, & Kapoor, 1989).

Our results with respect to polyphenol content are also in agreement with the finding of Osuntogun, Adewusi, Ogundiwin, and Nwasike (1989), who observed 20% reduction in total polyphenol content of Nigerian sorghum due to steeping. The decrease in the level of phytic acid and polyphenols during soaking may be attributed to their leaching in soaking water under the concentration gradient (Abdullah, Baldwin, & Minor, 1984) and endogenous phytase activity (Liang, Han, Nout, & Hamer, 2009). The lower level of total phenols and β -carotene after soaking may be due to release of phenolic compounds into the soaking water (Akillioglu & Karakaya, 2010).

The results of the present investigation of reduction in β -carotene content upon soaking are in agreement with Afify, El-Beltagi, El-Salam, and Omran (2012), who reported the reduction in antioxidant activity and antioxidant capacity after soaking due to leaching of total phenols, flavonoids, vitamin E, and β -carotene in soaking water.

The reduction in iron content during soaking might be due to leaching out of minerals in soaking water (Malik, Singh, & Dahiya, 2002). Similar reduction was reported by Lestienne, Icard-Vernière, Mouquet, Picq, and Trèche (2005) in iron content of pearl millet grains after 24 h of soaking. Reduction in ash content during soaking may be due to the leaching of both micro and macro elements and anti-nutrients into the extracting medium (Mugendi et al., 2010).

3.2. Effect of pressure cooking

Phytic acid and total phenols reduced significantly ($p < 0.05$) after both 5 and 10 min of pressure cooking (Table 3). Pressure cooking led to a significant ($p < 0.05$) decrease in the β -carotene, iron, and ash contents of the pearl millet just after 2 min of cooking. In the present study, the soaking and cooking water were discarded and leaching may be the major reason along with thermal degradation for reduction in phytic acid, total polyphenols, β -carotene, iron and ash contents. Another reason attributed to the decrease of these compounds is their thermal degradation during pressure cooking (Kataria, Chauhan, & Punia, 1989). Bishnoi, Khetarpaul, and Yadav (1994) found that domestic processing and cooking methods reduced the phytic acid and polyphenol contents of various pea varieties, with germination for 48 h having a marked lowering effect. Vijayakumari, Siddhuraju, and Janardhanan (1996) studied the effect of soaking, cooking, and autoclaving on the concentrations of phytic acid in the tribal pulse *Mucunamono sperma* and found that cooking for 3 h resulted in significant reductions, with even higher losses associated with autoclaving. The findings of the

Table 3. Effect of pressure cooking on anti-nutrients, β -carotene, iron and ash content of pearl millet

Time (min)	Phytic acid (mg/100 g)	Total polyphenols (mg/100 g)	β -carotene (μ g/100 g)	Iron (ppm)	Ash (%)
0	683.07 \pm 1.87 ^a	207.23 \pm 3.06 ^a	85.74 \pm 0.82 ^a	43.23 \pm 0.28 ^a	1.72 \pm 0.007 ^a
2	631.38 \pm 2.18 ^b	144.56 \pm 3.19 ^b	73.48 \pm 1.33 ^b	39.43 \pm 0.09 ^b	1.52 \pm 0.005 ^b
5	608.51 \pm 1.65 ^c	143.49 \pm 1.78 ^b	69.08 \pm 1.86 ^c	38.03 \pm 0.63 ^c	1.43 \pm 0.010 ^c
7	605.62 \pm 3.33 ^c	122.69 \pm 3.01 ^c	64.37 \pm 1.91 ^d	36.38 \pm 0.22 ^d	1.38 \pm 0.005 ^d
10	603.87 \pm 2.23 ^c	105.09 \pm 1.23 ^d	60.20 \pm 1.28 ^e	36.30 \pm 0.33 ^d	1.34 \pm 0.009 ^e

Notes: Data are presented as means \pm SEM ($n = 3$). Means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

present investigation in context to β -carotene are in agreement with the results obtained by Marty and Berset (1986), who reported that the degradation of β -carotene during cooking may be due to thermal stress.

Haytowitz and Matthews (1983) studied the effect of cooking on nutrient retention in legumes and found a significant amount of iron ions in cooking water showing leakage of iron complexes from chickpea into hot water. Increase in electrical conductivity of the cooking water studied by Avola, Patane, and Barbagallo (2012) also supports the leaching out of minerals during cooking treatment. Collective effect of leaching out of minerals and anti-nutritional factors from pearl millet grains might be the reason for reduction in ash percentage in the present investigation during pressure cooking treatment (Borade, Kadam, & Salunkhe, 1984).

3.3. Effect of steaming

The results of the present study revealed that phytic acid was reduced significantly ($p < 0.05$) from its initial value of 683.07 ± 1.87 to 625.34 ± 3.34 and 616.93 ± 1.76 mg after 5 and 10 min of steaming, respectively (Table 4). Similarly, total polyphenol content was also reduced significantly ($p < 0.05$) from its initial value of 207.23 ± 3.06 to 138.96 ± 2.34 mg, 124.03 ± 1.54 and 115.23 ± 2.28 mg after 5, 10, and 20 min of steaming, respectively. β -carotene and ash content were reduced significantly ($p < 0.05$) as the steaming time was increased and there were also significant ($p < 0.05$) losses of iron content after 5 and 15 min of steaming. In both the treatments, viz. pressure cooking and steaming, there was a definite reduction in all the studied parameters, but the reduction was more in pressure cooking than the steaming. This is attributed to the fact that pressure cooking is more severe heat treatment than steaming.

3.4. Effect of controlled germination

From Table 5, it is evident that the phytic acid, total polyphenols, iron and ash contents were reduced significantly ($p < 0.05$) as a result of malting just after 12 h and continued till the end of 52 h. However, β -carotene content losses were non-significant up to 46 h of malting and then reduced significantly after 46 h. The findings of the present investigation in context to phytic acid are in agreement with the results obtained by Gupta and Sehgal (1991). They observed a decrease in phytic acid contents of cereal grains meant for the production of weaning foods by soaking and germination. This decrease in phytic acid during soaking could be attributed to the leaching into soaking water under the concentration gradient. Another reason in phytic acid decrease during germination was attributed to the increase in the phytase activity in germinating grains (Borade et al., 1984; Rao & Deosthale, 1982). Phytase activity was also observed in germinating wheat, barley, rye, and oats, which hydrolyze phytate to phosphate and myoinositol phosphates (Larsson & Sandberg, 1992).

Our findings with reference to polyphenols are in accordance with the findings of Prasad, Alok, Arvind, and Nitya (2015) and Sharma and Sehgal (1992), who reported that germination of pearl millet reduces the polyphenol content. This loss of polyphenols during germination may be attributed to

Table 4. Effect of steaming on anti-nutrients, β -carotene, iron and ash content of pearl millet

Time (min)	Phytic acid (mg/100 g)	Total polyphenols (mg/100 g)	β -carotene (μ g/100 g)	Iron (ppm)	Ash (%)
0	683.07 ± 1.87^a	207.23 ± 3.06^a	85.74 ± 0.82^a	43.23 ± 0.28^a	1.72 ± 0.007^a
5	625.34 ± 3.34^b	138.96 ± 2.34^b	75.29 ± 1.19^b	39.45 ± 0.38^b	1.55 ± 0.004^b
10	616.93 ± 1.76^c	124.03 ± 1.54^c	71.90 ± 1.27^c	38.26 ± 0.11^{bc}	1.48 ± 0.006^c
15	611.97 ± 2.42^c	120.83 ± 1.91^{cd}	68.80 ± 1.11^d	37.18 ± 1.02^c	1.40 ± 0.010^d
20	611.35 ± 3.28^c	115.23 ± 2.28^d	63.51 ± 0.74^e	36.79 ± 0.55^c	1.36 ± 0.003^e

Notes: Data are presented as means \pm SEM ($n = 3$). Means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

Table 5. Effect of controlled germination on anti-nutrients, β -carotene, iron and ash content of pearl millet

Time (h)	Phytic acid (mg/100 g)	Total polyphenols (mg/100 g)	β -carotene (μ g/100 g)	Iron (ppm)	Ash (%)
0	683.07 \pm 1.87 ^a	207.23 \pm 3.06 ^a	85.74 \pm 0.82 ^a	43.23 \pm 0.28 ^a	1.72 \pm 0.007 ^a
12	619.91 \pm 2.65 ^b	196.56 \pm 3.39 ^b	82.82 \pm 0.68 ^{ab}	39.22 \pm 0.48 ^b	1.55 \pm 0.007 ^b
18	608.19 \pm 2.18 ^c	189.09 \pm 1.58 ^c	82.29 \pm 1.47 ^{ab}	38.99 \pm 0.15 ^b	1.52 \pm 0.008 ^c
24	591.53 \pm 3.55 ^d	182.96 \pm 2.55 ^{cd}	81.78 \pm 2.25 ^{ab}	38.64 \pm 0.41 ^b	1.50 \pm 0.006 ^d
36	582.35 \pm 2.43 ^e	180.83 \pm 2.23 ^d	81.68 \pm 2.13 ^{ab}	38.17 \pm 0.13 ^b	1.47 \pm 0.001 ^e
40	504.62 \pm 2.85 ^f	176.83 \pm 1.85 ^{de}	81.51 \pm 1.88 ^{ab}	38.01 \pm 0.79 ^b	1.44 \pm 0.002 ^f
46	444.10 \pm 4.22 ^g	173.89 \pm 3.22 ^e	81.34 \pm 2.22 ^{ab}	37.69 \pm 1.18 ^b	1.42 \pm 0.009 ^g
52	421.90 \pm 4.17 ^h	172.56 \pm 2.17 ^e	80.28 \pm 2.07 ^b	37.48 \pm 1.52 ^b	1.39 \pm 0.010 ^h

Notes: Data are presented as means \pm SEM ($n = 3$), Means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

the activation of polyphenol oxidase (Rao & Deosthale, 1982) and to the hydrolysis of tannin–protein and tannin–enzyme complexes which results in the removal of tannins or polyphenols (Farhangi & Valadon, 1981). In addition to leaching, other reason for the reduction of total phenols during germination could be facilitated by increased enzymatic hydrolysis (Bishnoi et al., 1994).

The findings of the present investigation in context to β -carotene are not in agreement with the results obtained by Yang, Basu, and Oraikul (2001), who reported that the concentration of β -carotene steadily increased in wheat with increase in germination time. Similarly, Lee, Hwang, Lee, Chang, and Choung (2013) reported that during germination, β -carotene content of soybean gets accumulated in whole soybean sprouts and cotyledon, while hypocotyls did not accumulate lipophilic pigments during germination. The contradictory finding of the present study may be attributed to the fact that grains were dried and cotyledons were removed where the major portion of β -carotene was lost.

Major reduction in iron content during malting was observed after 9 h of soaking treatment (Table 2). This reduction in iron content attributed to the soaking treatment given to the pearl millet grains before malting. The reduction in mineral contents during soaking and sprouting treatments might be due to the leaching out of minerals in the soaking water. These findings are supported by the observations recorded by earlier workers (Chavan, Kadam, & Beuchat, 1989; Rani & Hira, 1993).

Our results in relation to decrease in ash content (Table 5) reveals that ash content decreased significantly ($p < 0.05$) during controlled germination and reached to the tune of 1.39% in comparison to 1.72% at zero hour of germination. This reduction in the ash content of pearl millet flour may be attributed to the leaching out of solid matter during the soaking step prior to the malting of pearl millet. The findings of the present study are in agreement with the observations of other workers (Duhan, Khetarpaul, & Bishnoi, 1999; Gernah, Ariahu, & Ingbian, 2011; Mubarak, 2005) who reported that germination and cooking processes cause a significant decrease in ash content.

3.5. Effect of combination of processing treatments

As evident from the results discussed above, different treatments lead to the reduction of anti-nutrients to different extents. Hence, the combinations of different processing treatments were also studied to optimize the best treatment combination to obtain pearl millet flour suitable for the preparation of various products such as ready-to-reconstitute weaning food. It can be noted here that out of the two heat processing treatments discussed above (Pressure cooking and steaming), pressure cooking was selected for making further combinations because of its significant effect on anti-nutrients' reduction in lesser time as compared to the steaming. As the purpose of the work was to

prepare flour suitable for various food products, therefore, further two treatments (pearling and extrusion) were also incorporated in the study. It is evident from Table 6 that phytic acid was reduced significantly ($p < 0.05$) in all the three combinations in comparison to the control. Data indicated that reduction in phytic acid content in comparison to control was 36, 37.3, and 38% in combination (A), (B), and (C), respectively. However, the effect of treatment (B) and (C) on the reduction of phytic acid content was non-significant. The results obtained in the present study are in agreement with the results obtained by Duhan et al. (1999), who subjected Manak, a high-yielding cultivator of *Cajanus* (Pigeon pea) to various domestic processing and cooking methods including soaking, soaking and dehulling, ordinary cooking, pressure cooking and germination, and found that the phytate concentration was reduced significantly. A reduction of 13–35% was observed after extrusion of a wheat bran-starch-gluten mixes (Andersson, Hedlund, Jonsson, & Svensson, 1981). Reduced phytate levels in wheat flour were also reported by Fairweather-Tait, Portwood, Symss, Eagles, and Minski (1989) on extrusion cooking. This reduction was attributed to high shear coupled with very high temperature during extrusion processing, which hydrolyzed phytate to release phosphate molecules.

Total phenols were also reduced significantly ($p < 0.05$) with respect to the control as a result of treatment (A), (B), and (C), but the extent of reduction between combination (B) and (C) was non-significant (Table 6). Results indicated that reduction in total phenol content in comparison to control was 37.8, 26.4, and 28.6% in combination (A), (B), and (C), respectively. Our findings in context to total phenol reduction are in agreement with findings of Sinha and Kawatra (2003). They also reported significant reduction in the concentration of phytic acid and polyphenols in cowpea as a result of soaking, de-hulling, ordinary cooking, pressure cooking and germination. Changes in the polyphenol content after thermal treatment might result in the binding of phenolic compounds with other organic materials present (Alonso, Rubio, Muzquiz, & Marzo, 2001). β -carotene was also lost significantly ($p < 0.05$) in each treatment as compared to control (Table 6). The reduction during extrusion cooking may be attributed to the exposure of β -carotene to high temperatures and high mechanical stresses accelerating oxygen or light induced as well as other chemical reactions or structural changes (Emin, Mayer-Miebach, & Schuchmann, 2012).

Iron was reduced significantly ($p < 0.05$) in both the treatments (A) and (B). Data revealed that iron content was reduced to the tune of 24.7 and 22.1% in treatments (A) and (B), respectively. The decrease in iron content during treatments (A) and (B) may be attributed to the leaching out of minerals in the spent water during soaking. However, in combination (C), the iron content remained almost unaffected (42.06 ppm) and was comparable to that of control sample (43.23 ppm). As compared to other treatments, higher value of iron in treatment “C” could be attributed to the minute addition of iron from the screws and inner parts of extruder during processing. Our findings are supported by Alonso et al. (2001) and Singh, Chauhan, Suresh, and Tyagi (2000), who reported a slight increase in iron content in extrusion

Table 6. Effect of combination of processing treatments on anti-nutrients, β -carotene, iron and ash content of pearl millet

Treatments	Phytic acid (mg/100 g)	Total polyphenols (mg/100 g)	β -carotene (μ g/100 g)	Iron (ppm)	Ash (%)
Control	683.07 \pm 1.87 ^a	207.23 \pm 3.06 ^a	85.74 \pm 0.82 ^a	43.23 \pm 0.28 ^a	1.72 \pm 0.07 ^a
A*	437.42 \pm 2.08 ^b	129.00 \pm 1.16 ^c	85.74 \pm 0.82 ^a	32.54 \pm 0.39 ^b	1.31 \pm 0.06 ^b
B**	428.24 \pm 2.09 ^c	152.44 \pm 1.54 ^b	78.75 \pm 1.21 ^b	33.67 \pm 0.14 ^b	1.35 \pm 0.008 ^b
C***	423.55 \pm 1.64 ^c	148.14 \pm 1.89 ^b	72.81 \pm 1.45 ^c	42.06 \pm 0.46 ^a	1.33 \pm 0.005 ^b

Notes: Data are presented as means \pm SEM ($n = 3$), Means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

*A = 9 h soaking + 40 h controlled germination + 5 min pressure cooking.

**B = 9 h soaking + 40 h controlled germination + pearling.

***C = 9 h soaking + 40 h controlled germination + pearling + extrusion cooking.

probably due to the addition of these minerals through water used during extrusion processing and also due to the wear of metallic pieces of the extruder. Similarly, Camire (2000) also noted that total iron content was increased by 38% due to extrusion.

It is evident from Table 6 that ash content of pearl millet was reduced significantly ($p < 0.05$) as a result of treatment (A), (B), and (C) in comparison to control. However, the extent of reduction amongst treatments (A), (B), and (C) was non-significant. The most probable reason of reduction in ash content may be attributed to the collective action of mechanical stress, heat degradation, and leaching out of minerals and anti-nutrients during different treatments. The other reason in the reduction of ash content is the removal of mineral-rich outer greyish layer or bran portion of the pearl millet during pearling (El Hag, El Tinay, & Yousif, 2002).

From the data represented in the Table 6, it is evident that there is significant ($p < 0.05$) reduction in both the anti-nutrients, viz. phytic acid and total polyphenols in treatment combinations (A) and (C) as compared to control. Table 6 also suggests that the iron content in treatment combination (C) remained similar to that of control, however, in case of combination (A), it was less than the control. Therefore, looking into the effect of treatments (A) and (C) on the reduction in anti-nutrients in pearl millet, both the treatments were selected for the preparation of pearl millet flour.

4. Conclusions

It can be concluded that domestic processing treatments, viz. soaking, steaming, pressure cooking, malting, pearling, and extrusion, could reduce the level of anti-nutrients significantly. Out of soaking, pressure cooking, and steaming, higher reduction in anti-nutrients as well as iron and β -carotene were observed in pressure cooking. However, controlled germination was found to be the most suitable treatment which resulted in maximum loss of anti-nutrients with no significant reductions in iron and β -carotene content. Effect of combination of different methods showed that treatment "C" (9 h Soaking + 40 h Controlled Germination + Pearling + Extrusion cooking) was comparatively better in terms of iron and phytic acid. Overall, it can be concluded that combination of domestic treatments is a better approach rather than individual process in improving the nutritional profile of pearl millet, which can be consumed directly or can be used as one of the ingredients for formulations like weaning foods, bakery products, etc.

Funding

This work is a part of the National Agricultural Innovation Project (NAIP) entitled "A Value Chain on Composite Dairy Foods with Enhanced Health Attributes" [Sub-project code C-1300] funded by Indian Council of Agricultural Research (ICAR), India.

Competing interests

The authors declare no competing interest.

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Citation information

Cite this article as: Effect of domestic processing treatments on iron, β -carotene, phytic acid and polyphenols of pearl millet, Manvesh Kumar Sihag, Vivek Sharma, Ankit Goyal, Sumit Arora & Ashish Kumar Singh, *Cogent Food & Agriculture* (2015), 1: 1109171.

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