International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 1041-1045

Received: 10-10-2020
Accepted: 17-11-2020

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Combination of air plasma bubbling and soaking in the reduction of phytic acid and improving free iron availability in pearl millet

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DOI: https://doi.org/10.22271/chemi.2021.v9.i1o.11361

Abstract
Pearl millet was subjected to air plasma bubbling along with soaking to enhance HCl extractable iron by reducing phytic acid content. The pearl millet was soaked for 11 h and 10 h followed by plasma bubbling (1 and 2 h) at input voltage 180 V and 10 lph flow rate. A 64.9% and 70.93% of reduction in phytic acid was observed with treatments. The total iron content was reduced while HCl extractable iron content improved by 68% and 59% with 1 h and 2 h exposure time. The significant changes ($p \leq 0.05$) in pearl millet's physical, nutritional, and techno-functional properties were noticed with plasma exposure. The present study shows the potential of plasma exposure along with soaking for reducing phytic acid content, improving free iron, and ameliorating techno-functional properties of pearl millet.

Keywords: Pearl millet, iron, phytic acid, air plasma bubbling

1. Introduction
Pearl millet is one among the most cultivated grains and stands fourth among the world tropical cereal grains (Sene *et al*. 2018) [33]. Two predominant pearl millet producing areas are Asia and Africa (Jukanti *et al*. 2016) [10]. Pearl millet is drought and heat tolerant crop which can adapt to a various environmental conditions (Sene *et al*. 2018) [33]. Pearl millet has excellent nutritive value with high contents of fat, protein, and minerals in comparison with other major cereals like wheat, rice, and maize. The pearl millet protein comprises lysine and methionine (>40%), threonine (>30%) than maize protein (Krishnan and Meera 2018; Sene *et al*. 2018) [33, 13]. It composed 4-39.71 mg/100 g of iron content based on the cultivar and grain fractions (Krishnan and Meera 2018; Tripathi and Platel 2013) [13, 38]. Though it is rich in iron, the bioaccessibility of iron in pearl millet is merely 0.39 mg/100 g, due to the presence of anti-nutrient phytic acid (Tripathi and Platel 2013) [38]. Thus, it is essential to decrease lessen the phytic acid to improve the free and extractable iron, which possess high absorption in the body (Krishnan and Meera 2018) [13]. The accumulation of phytic acid primarily occurs in the aleurone layer of pearl millet (Jukanti *et al*. 2016) [10]. It can bind with multivalent minerals such as iron which aids in lowering their bioavailability (Kumari *et al*. 2015) [15]. So, it is essential to explore the new techniques which enhance the phytic acid reduction and enhances mineral availability.
Cold plasma is one of the nonthermal strategies picking up significance in these days particularly in food applications. As a result of its exceptionally receptive and self-quenching nature, the plasma responsive species have the possibility to address safety, nutrition and quality issues of food (Perinban *et al*. 2019; Potluri *et al*. 2018) [25, 26]. The atmospheric plasma is primarily composed of oxygen (O^-, O^+, O_2^-, O_3) and nitrogen (N^+, N_2^-, NO^+) reactive species (Ratish Ramanan *et al*. 2018) [28]. These active plasma species are capable of inducing changes in morphology, composition and functionality of the product (Misra *et al*. 2018) [20]. At the point when the air plasma is bubbled into the water the reaction of plasma receptive species with fluid medium electrolyzes the water particles. It then forms several plasma active species including acids such as nitrous acid, nitric acid, hydrogen peroxide. This brings down pH and elevates conductivity of the fluid medium which thusly improves the impact of plasma species on the sample (Perinban *et al*. 2019; R. Zhou *et al*. 2018) [25, 42].

In the present work, plasma was given in combination with soaking to improve its adequacy on the pearl millet. The phytic acid, total and HCl extractable iron along with changes in the physical, chemical, and techno-functional properties of the pearl millet with treatments were analyzed.

2. Materials and Methods

2.1 Sample collection

Pearl millet was obtained from supermarket (Thanjavur, Tamilnadu) was cleaned by setting them free from dust and foreign materials. The fresh sieved pearl millet was filled in polyethylene covers and stored at ambient temperature (30±2 ^{O}C).

2.2 Air plasma bubbling system

An indigenously developed plasma bubbling system of DBD type (dielectric barrier discharge) was used for treating pearl millet (Aparajhitha and Mahendran 2019) [3]. The atmospheric air was employed as the input gas. The flow rate and voltage of plasma were controlled by the voltage regulator. Pearl millet was exposed to an input voltage of 180 V with an airflow of 10 liters per hour for different exposure times. The airflow rate and voltage used in the current study were optimized already by Aparajhitha and Mahendran (2019) [3].

2.3 Sample preparation and treatments

The sample without any treatment was considered as control (C). A 12 h soaking period was considered as standard pre-treatment time for grains (Dipnaik and Bathere 2017) [5]. By taking this in consideration the total treatment time was fixed as 12 hours. The pearl millet was soaked for 11 h followed by plasma bubbling for 1 h denoted as T1, and the sample soaked for 10 h along with plasma bubbling (2 h) was indicated as T2. The water was drained from the pearl millet grains was dried at 60 °C for 30 min. The pearl millet samples were analyzed for phytin, total iron, free iron, physicochemical and techno-functional properties.

2.4 Estimation of phytic acid content

The phytic acid content of samples was assessed using a spectrophotometer by adopting Wheeler and Ferrel R (1971) [41] method. The phytic acid in the pearl millet was extracted by trichloroacetic acid and precipitated in the form of ferric iron. Phytate phosphorous content in the pearl millet was estimated from ferric iron content by presuming a 4:6 molar ratio.

2.5 Total iron and free iron determination

Total iron in the pearl millet was extracted by wet-acid digestion method using the mixture of nitric acid: perchloric acid and estimated using the atomic absorption spectroscopy (Kumar and Chauhan 1993) [14].

The acid extractable iron content was measured by extracting iron in HCl of 0.03 N (similar to the concentration observed in the human stomach). The extract was filtered with the Whatman No. 42 filter paper and oven-dried at temperature of 100° C followed by wet digestion. Atomic absorption spectroscopy was used to analyse free iron in the digested sample (Sokrab *et al*. 2014) [35].

2.6 Estimation of physical properties

The color (L^*, a^*, b^*) of the pearl millet sample were obtained by Hunter lab colorimeter (Colourflex EZ model: 45/0 LAV). The color intensity (ΔE) was estimated by substituting in the below equation 1.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \text{ ---------- (1)}$$

The bulk density of the pearl millet sample was estimated by recording the weight of a known volume of the sample according to Falade and Kolawole (2013) [8].

2.7 Analysis of chemical properties

The protein, fat, crude fiber, and ash of the pearl millet treatments were analysed according to AOAC (2010) [2]. The moisture content was measured by moisture meter (Mettler Toledo HE53, Greifensee, Switzerland). Carbohydrate content was estimated by substituting in below equation 2.

100 - (Crude fat + crude fiber + crude protein + ash + moisture) ---------- (2)

The reducing sugar content was measured by employing McCleary and McGeough (2015) [19] method.

2.8 Analysis of techno-functional properties

The pearl millet samples were milled into a fine powder to particle size of 0.5 microns and used for analyzing techno-functional properties. Water absorption, oil absorption, and dispersibility were analyzed by adopting Ramashia *et al*. (2017) [27] method. The water solubility and swelling capacity of the pearl millet samples were analysed based on Kusumayanti *et al*. (2015) [16] procedure.

2.9 Statistical analysis

All the analysis was done in triplicate and the obtained data was expressed as mean ± S.D. Data analysis was performed using SPSS (IBM statistical analysis, version 20.0). DMRT (Duncan's multiple range test) was used to determine significant difference among the treatments using one-way ANOVA (analysis of variance) test with a 5% significant level.

3. Result and Discussion

3.1 Phytic acid content of the pearl millet samples

The content of phytic acid in the control was 369.37 mg/100 g. The variations in phytic acid content with soaking and plasma bubbling was abridged in table 1. A 64.9% and 70.93% decrement in phytic acid was noticed in T1 and T2. The reduction with soaking and plasma bubbling is due to improvement of the phytase enzyme activity. The bubbling of plasma into the sample results in rapid hydrolysis and faster imbibition of water into millet resulting in enhanced phytase activity. This leads to the elevation of the phytic acid degradation to its derivative forms by breaking down the bonds with minerals and thus improving their bioavailability. This similar kind of increment in phytase enzyme activity was observed by Sadhu *et al*. (2017) [29] in cold plasma exposed mung beans.

More reduction in phytin content was obtained with plasma-treated air bubbling when compared with other techniques like soaking (15 to 30%) microwave (33.4% to 35.5%), ultrasound (18.2 to 30.7%), and high-pressure processing (36 to 45.5%) (Deng *et al*. 2015; Ertas 2013; Linsberger-Martin *et al*. 2013; Olika *et al*. 2019; Sene *et al*. 2018) [4, 7, 18, 22, 33].

3.2 Total and free iron content

The alterations in the free and total iron content of the pearl millet with given treatments are represented in Figure 1. Total iron in control was obtained to be 39.92 mg/100 g of which 12.6% was in extractable form. The soaking and plasma

exposure treatments reduced the total content to 28.71 and 28.09 mg/100g. The leaching of iron contents into the soaked water leads to the reduction in total iron content among treated samples. A similar decrement in total iron content among soaked grains was reported in several studies (ElMaki *et al*. 2007; Lestienne *et al*. 2005; Saharan *et al*. 2001) [6, 17, 30]. The extractability of iron was increased with soaking and plasma bubbling treatments to 68% in T1 and 58% in T2. Low extractability in control was due to the antinutrient phytic acid (Kumari *et al*. 2015) [15]. Reduction in contents of phytic acid among the treated samples improved the free iron content which enhances the iron bioavailability (Sadhu *et al*. 2017) [29]. The decrement in the extractability of iron with exposure time in T2 possibly due to leaching of the available free iron into water or due to development of complexes with other compounds.

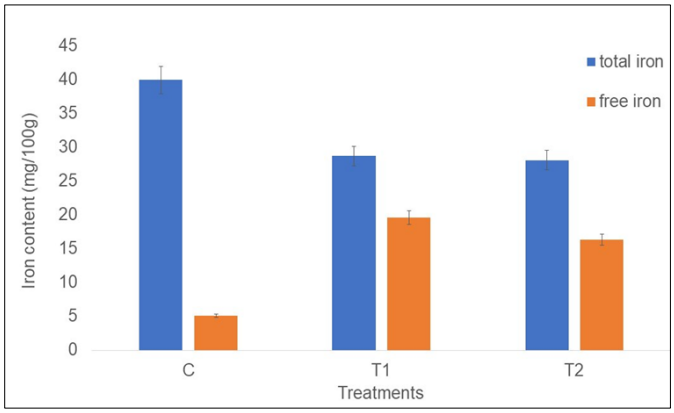

Fig 1: The effect of air plasma bubbling in combination with soaking on the total and free iron content of pearl millet. Vertical bars represent the standard error of the obtained values.

Treatments: C-control, T1- 11 h soaked and 1 h air plasma bubbling, T2- 10 h soaked and 2 h air plasma bubbling.

3.3 Changes in chemical properties

Variations in the nutritional properties of the pearl millet with given treatments were mentioned in Table 1. The control C has protein content of 11.09%, which decreased to 10.27% initially in T1 and increased to 11.8% with treatment time T2. Free radicals and reactive species in plasma increase the non-protein nitrogen content resulting in an increment in protein. A similar increment in protein was perceived by Thirumdas *et al*. (2015) [36] with exposure time in plasma-treated basmati rice. No significant changes were noticed in crude fat content with the given treatments. Whereas reduction in carbohydrate content observed among treated samples from 69.44% in control to 69.29 and 68.36% in T1 and T2 respectively. The decrement possibly because of leaching of soluble carbohydrates into the soaked water during plasma bubbling. The leaching out of these soluble starches water leads to penetration of soaked water into pearl millet samples (Pankaj *et al*. 2018) [24]. As an outcome, the moisture content was improved with the treatment time in pearl millet from 10.09% in control to 10.65% and 10.78% in T1 and T2 respectively.

Crude fiber content was observed as 2.09% in control. It was improved by 2.58% at the initial stages of treatment in T1 whereas a reduction of 1.89% occurred with treatment time in T2. The decrement with treatment time is due to the degradation of fibrous structures by the action of highly reactive plasma spices (Obadina *et al*. 2017; Vasishtha and Srivastava 2013) [21, 40]. Plasma exposure improved the ash content from 1.55% in C to 1.20% and 1.25% in T1 and T2. The reactive species and free radicals in plasma increase the ash content of the sample. Thirumdas *et al*. (2015) [36] also reported a similar result of ash content in plasma-treated basmati rice. The reducing sugar percentage was improved from control 1.02% to 4.72% and 4.07% in T1 and T2. The improvement of reducing sugars with exposure time is due to high energy plasma species which aids in depolymerization of starch (Almeida *et al*. 2015; Sarangapani *et al*. 2016; Y. Zhou *et al*. 2018) [42]. The reduction was observed in T2 in contrast with T1 is because of the discharge of simple sugars into the soaked water (Urga and Gebretsa 1993) [39].

3.4 Effect of given treatments on physical properties

Alterations in the color and bulk density with the treatments were given in Table 1. The changes in Δ E (colour intensity) of the pearl millet were noticed with the plasma exposure. The bubbling action of plasma results in leaching of pigments into soaked water. The bulk density of the pearl millet was reduced with the given treatment from 0.78 kg/ m^3 in C to 0.75 and 0.73 kg/ m^3 in T1 and T2. Bulk density of the sample is negatively correlated with its moisture content (Handa *et al*. 2017) [9]. Since the increment in moisture was discerned with given treatments, the bulk density of samples was reduced.

Table 1: The effect of air plasma bubbling with soaking on phytic acid and physico-chemical properties of pearl millet

Parameters	C	T1	T2
Phytic acid (mg/100 g)	369.37 ± 17.19 ^{c}	115.35 ± 13.43 ^{b}	95.65 ± 10.3 ^{a}
Protein (%)	11.09±0.00^{b}	10.75±0.06^{a}	11.82±0.07^{c}
Fat (%)	5.72±0.06^{a}	5.51±0.17^{a}	5.87±0.17$^{a, b}$
Carbohydrate (%)	69.44±0.10^{b}	69.29±0.07^{b}	68.36±0.08^{a}
Moisture (%)	10.09±0.02^{a}	10.65±0.12^{b}	10.78±0.02^{c}
Crude fiber (%)	2.09±0.06^{b}	2.58±0.03^{c}	1.89±0.03^{a}
Ash (%)	1.55±0.02^{c}	1.20±0.00^{a}	1.25±0.00^{b}
Reducing sugars (%)	1.02±0.01^{a}	4.72±0.06^{c}	4.07±0.06^{b}
Δ E	-	2.34±0.11^{a}	1.99±0.06^{b}
Bulk density (kg/ m^3)	0.78±0.00^{c}	0.75±0.00^{b}	0.73±0.01^{a}

The data mentioned above were expressed in mean ± standard deviations of triplicate samples (on a dry weight basis). Different superscript letters in each row differ significantly from each other ($p< 0.05$), as evaluated by Duncan's multiple range test. Treatments: C-control, T1- 11 h soaked and 1 h air plasma bubbling, T2- 10 h soaked and 2 h air plasma bubbling.

3.5 Effect of given treatments on techno-functional properties

The variations in techno-functional properties with soaking and plasma bubbling are represented in Table 2. Alterations in the starch and protein structure of sample reflects on its water and oil absorption (Kajihausa *et al*. 2014) [11]. Given treatments improved the water absorption and oil absorption of the samples in T1 and T2 by 29% and 37% in comparison with control. Plasma treatment improves the number of active sites by inducing cross-linkages in starch and also alters protein's secondary structure leading to increment in water absorption and an oil absorption of the sample (Pal *et al*. 2016; Thirumdas *et al*. 2017) [29]. The swelling capacity of treated pearl millet increased by 8% in T1, whereas plasma exposure time reduced swelling capacity by 13% in T2. The loosening or damage of the structural compounds due to plasma active species causes a decrement in T2. Similar results were obtained by Sarangapani *et al*. (2017) [32] in plasma-treated parboiled rice. The solubility of treated

samples enhanced by 18 and 28% in T1 and T2 in contrast with C. These changes were identified due to the damage of complex structures to soluble components like amylose and amylopectin by the action of plasma reactive species (Kim *et al*. 2019; Sarangapani *et al*. 2016) [31]. Particle size of the flour impacts the dispersibility of the sample. Since the soaking combined with plasma exposure resulted in more reduction of particle size (Silveira *et al*. 2019) [34], dispersibility of pearl millet increased by 77 and 79% in comparison with control.

Table 2: The effect of air plasma bubbling in combination with soaking on techno-functional properties of pearl millet

Parameters	C	T1	T2
Water absorption (ml/g)	1.11±0.02^{a}	1.44±0.02^{b}	1.53±0.02^{c}
Oil absorption (ml/g)	1.06±0.00^{a}	1.28±0.00^{b}	1.41±0.02^{c}
Swelling capacity (g/g)	0.23±0.00^{b}	0.25±0.01$^{b, c}$	0.20±0.02^{a}
Solubility (g/g)	1.81±1.53^{a}	2.15±1.31^{b}	2.32±1.51^{c}
Dispersibility (%)	72.16±0.28^{a}	77.83±0.76^{b}	79.5±0.50^{c}

The data mentioned above were expressed in mean ± standard deviations of triplicate samples (on a dry weight basis). Different superscript letters in each row differ significantly from each other ($p< 0.05$), as evaluated by Duncan's multiple range test.

Treatments: C-control, T1- 11 h soaked and 1 h air plasma bubbling, T2- 10 h soaked and 2 h air plasma bubbling.

4. Conclusion

The air plasma bubbling along with soaking is an effective technique in reducing the phytic acid by improving free iron content. A higher reduction (70.93%) of phytic acid content was noticed at 2 hr bubbling with 10 h soaking and greater HCl extractable iron content (68%) was noticed at 1 h bubbling with 11 h soaking. The significant variations ($p \leq 0.05$) were observed in color, bulk density, carbohydrate, reducing sugars, protein, ash, moisture, fiber, water and oil absorption, swelling capacity, solubility, and dispersibility of treated pearl millet. In conclusion, plasma bubbling under optimized conditions can improve the bioavailability of the minerals by reducing the anti-nutrients and providing better quality.

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