Journal of Phytology 2020, 12: 62-66
doi: 10.25081/jp.2020.v12.6384
https://updatepublishing.com/journal/index.php/jp/

Research Article

ISSN: 2075-6240

Impact of soaking, sprouting on antioxidant and anti-nutritional factors in milletgrains

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Received: August 01 2020
Revised: September 18 2020
Accepted: September 21 2020
Published: Sepember 26 2020

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ABSTRACT

Commonly consumed millet types *viz*., Little millet, foxtail millet, pearl millet, finger millet, and Kodo millet were tested under two processing methods of soaking and germination at a different time point. Most popular processing practices adopted by Indian households were studied for their influence on the biochemical properties, antioxidant profile, and anti-nutritive factors. The results showed that the sprouting process showed a maximum influence on the antioxidant and anti-nutritive factors. The comparative analysis of the five millets suggested 24h soaking and 24h germination was found to be best for producing nutritionally enriched millet products. Tannin content decreases with an increase in germination. The reduction in tannin was about 50% in little millet (0.347 mg/g) and the highest was recorded in finger millet (2.07 mg/g). The highest amount of saponin content was found in pearl millet(39.53 mg/g) followed by finger millet (34.86 mg/g) with 24 hr soaking and 24 hr germination. The phytase activity was found to be higher in little millet (61.520 u/kg) when prolonged soaking and germination. The DPPH assay showed sprouted foxtail millet grains contain more antioxidant activity (81.13%) with extended period of soaking and germination.

KEYWORDS: Millets, tannin, saponin, phytase, antioxidants

INTRODUCTION

Millets are also considered to be a rich source of carbohydrates, phytochemicals, and also for other micronutrients like iron and zinc [1]. Especially millets are preferred for its malting quality which inturn enhances the bioavailability of the nutrients and also improves the nutritional quality. The amino acid constitution of the millet protein isa good source for methionine except for lysine and threonine. The availability of iron content in millets is high but the rate of bioavailability is less due to the presence of phenol fraction in the bran. Effective iron utilization in millets can be withheld by practicing traditional household processing techniques such as threshing, dry milling, dehulling, cleaning, soaking, germination which could reduce the effect of inhibitors [2]. These practices also decrease the effect of undesirable compounds, which increases the acceptability and nutritional quality [3]. Recently, processing, nutritional composition and health benefits of finger millet was studied and revealed that the process of pre soaking and germinating increases the dietetic and sensory properties of finger millet and equally reduces the anti nutritional and inhibitory activity of phenol, tannin and phytic acid [4]. In order to improve the bioavailability of the minerals, soaking is the best practice for popular food preparation which reduces the anti-nutritional factors such as phytic acid. The whole and dehulled seeds and flours of the millets were studied for its iron and zinc concentration and also for the degradation and leaching of phytate compounds after soaking for certain hours. The results revealed that the dehulled and milled millet grains before soaking facilitates leaching of phytates and phytases and hence phytate degradation happens. Germination is one of the processes practiced in legumes and cereals to increase their palatability and nutritional value via the breakdown of antinutrients such as phytate and protease inhibitors. Nutritionalcontent and functionality of millet grains nutrients were widely studied [5]. Many of the malted millet foods the grains are germinated and dried which leads to a lot of biochemical changes in nutrient composition. The grain nutrient analysis will give better information on the process of germination of seeds. Hence the present study was taken up to analyze antioxidant and anti-nutritive factors during the process of seed soaking followed by sprouting in popular millets viz., pearl millet, foxtail millet, little millet, Kodo millet, and finger millet

MATERIALS AND METHODS

The Pearl millet (CO 9), Kodo millet (CO (VK)2), Finger millet, Little millet (ALT1), and Foxtail millet (ALT1) were collected from Centre of Excellence, Millets, Athiyanthal, TNAU, Tiruvannamalai. All the grains were dehusked without any thermal or chemical treatment and were stored inan airtight container at room temperature.All the millet samples of 10 g soaked in 50 ml distilled water. The soaking for two time periods (12h and 24h), germinated and harvested for two time periods (12h, 24h). Dried, powdered samples were stored in airtight containers and used for analysis. The antioxidant activityand anti-nutritive factors were analyzed in different millets and at different time points. The amount of respective biochemical constituent is expressed in mg/g of sample. The non-enzymatic antioxidant assay of total phenols [6-10], DPPH assay, Tannin estimation, and phytate enzyme assay [11] were analyzed in the samples at different time points of soaking and germination. The experiments are carried out in a Completely Randomized Design (CRD). The data were analyzed by a two-way ANOVA table and the difference existed between the mean values are compared at the level of $P \leq 0.05$ through AgRes software.

RESULTS AND DISCUSSION

Millets are generally poor in digestibility and low bioavailability of minerals due to the presence of heritable anti-nutritional factors [12]. Processing operation reduces the anti-nutritional factors but increases the loss of micronutrients which are of major concern. Techniques like mechanical, thermal, or biological processes can improve the nutritional availability in foods [13,14]. Wet processing such as soaking germination and fermentation tends to reduce the phytic acid and increases the solubility of the minerals and thus enhances the bioavailability of minerals in cereals and legumes [15]. Processing value of millets in product development has a promising prospect with respect to the nutrition, quality, and health benefits which can be an alternate source to cereals but the scope and utilization are yet to be established [5].

The household processing technique such as pre-soaking and germination etc. are the best tool to enhance the level of nutrition content of food stuffs. Among many techniques, pre soaking and germination are the biochemical enrichment tool where it increases the bioavailability of minerals through transition of a seed from its dormant to vital active state which improves the nutritive level of seeds. Also reduces the antinutrients viz., oxalic acid, phytic acid, trypsin inhibitor and tannin as well as increases the optimum level of absorbable nutrients [16,17]. Thus in this present study, the influence of soaking and germination on biochemical constituents suchasenzymic and non-enzymic antioxidants, and anti-nutritive factors were investigated in the Little millet, foxtail millet, pearl millet, finger millet, and Kodo millet. The grains were soaked and germinated for different time intervals (Figure 1). Then the grains from each treatment were dried, powdered, and used for assessment of certain biochemical, antioxidant, and anti-nutritive factors.

The ash content has several variations in which 12h soaking 24h germinated millets showed loss of minerals by low ash content, whereas 24h soaking 24h germinated millets has a considerably high level of ash content, which is due to a decreased level of crude fat and carbohydrate. Moisture content increases with an increase in germination. When compared to other millets finger millet possesses the highest amount of moisture content. It is found that the moisture content of the seeds during germination is increased which means it absorbs water from soaking medium for the metabolism to initiate the immobilisation of amylase to the embryonic region which affects the structure of grain.

CHANGES IN LEVELS OF ENZYMIC ANTIOXIDANT

DPPH assay was conducted to analyze the changes in levels of enzymic antioxidants in selected millet. Both raw and sprouted millets contain enzymic antioxidant activity, in which foxtail millet was noted to have the highest amount ranging from 62.833±0.289% in T1 to 81.123±0.125% in T7, this is followed by finger millet, little millet, kodo millet and pearl millet (Table 1).

There is an increase in the antioxidant level in all control and treated millets. From table 1it is clear that among control, pearl

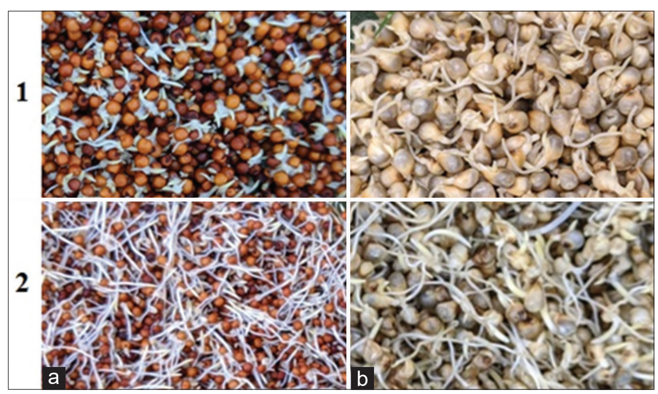

Figure 1: Soaking and Germination of Finger millet (a) and Pearl millet (b). Treatment for 12 hrs (1) and 24 hrs (2)

Table 1: Influence of soaking and germination time on DPPH content of the selected millets

Treatment	DPPH activity (%)				
	M1(1)	M2 (2)	M3 (3)	M4 (4)	M5 (5)
T1	28.077±0.067	62.833±0.289	56.713±1.027	34.123±0.214	24.863±0.228
T2	34.203±0.188	66.170±0.652	58.457±0.791	36.800±0.755	37.203±0.263
T3	42.913±0.878	70.917±0.936	63.133±0.709	42.873±0.860	40.440±0.383
T4	41.173±0.657	67.913±0.878	65.247±0.329	40.407±0.352	43.550±0.482
T5	50.333±0.577	75.740±4.019	68.153±0.172	50.040±0.069	53.080±0.069
T6	50.707±0.615	81.813±0.497	68.250±0.433	50.300±0.520	50.040±0.069
T7	55.550±0.638	81.123±0.125	78.340±0.489	56.167±0.144	62.433±0.451

(1) Finger millet; (2) Foxtail millet; (3)Kodo millet; (4) Little millet; (5) Pearl millet

millet (24.863±0.228 mg/g) has the lowest range, whereas, among treatments, finger millet T2 has the lowest range of enzymic antioxidant activity.The reducing ability of a substance may explain its potential antioxidant activity [18, 19]. The result from this present study showed that foxtail and Kodo millet possess the ability to scavenge free radicals and therefore can be employed as a source of antioxidant to prevent the accumulation of unwanted substances in the system [20].

From the treatments studied T7 recorded the highest antioxidant activity in all the millets. A similar study reported that the DPPH scavenging ability of the foxtail millet increased from 35.44 to 63.07mM trolox/g at the time of germination may be due to increased concentration of hexadecanoic acid methyl ester; 9,12-Octadecadienoic acid ethyl ester and synthesis of new polyphenolic compounds like pentadecanoic acid; 14-methyl methyl ester; 9,12,15Octadecatrienoic acid.During germination some bound components might have liberated and played a role in DPPH and hydrogen peroxide scavenging when the endosperm has modified by the hydrolytic enzymes [21]. Similar results are in accordance with [22].

SAPONIN CHANGES IN MILLETS

In the present study, pearl millet recorded the highest amount of saponin followed by foxtail millet, little millet, finger millet, and kodo millet. Increment of saponin content ranged from 29.117±0.732 to 39.530±0.148 mg/g in pearl millet and 24.730±0.204 to 34.860±0.079 mg/g in finger millet flour. This result may be due to the displacement of stored phytochemicals from the sprouts. Similarly from the other studies, saponin content was increased from 18.01 to 31.91 mg/g in finger millet [23]. Saponin is useful for the human physiological system and also for treating various diseases. Decreased in overall blood cholesterol is associated with the indigestion status of saponin. The abundant nature of phytochemicals in the millets increases the nutraceutical potentials, thus making them a reliable source of functional food [24].

Among treatments, it was observed that T7 (24h soaking 24h germination) has the highest range followed by T5 (24h soaking 12h germination) and T6 (12h soaking 24h germination), T4 (12h soaking 12h germination), T3 (24h soaking), T2 (12h soaking), T1 (control). When grains or seeds are hydrated (soaked) and then held (sprouted) under ambient conditions, both endogenous and newly synthesizing enzymes begin to modify seeds and increases the phytochemical constituents present in the millets. This is one of the reasons why the saponin content is high during germination than other treatments [25].

From the graph given below (Figure 2), it is clear that millet 5 with treatment 7(Pearl millet with 24h soaking 24h germination) shows the best performance which may be due to the activation of the endogenous enzyme in pearl millet. This is followed by M5T6, M5T4, M5T5, M5T3, M1T7. Next to pearl millet, finger millet with 24h soaking and 24h germination has a high amount of saponin. Among all the treatments, T7 has the best performing

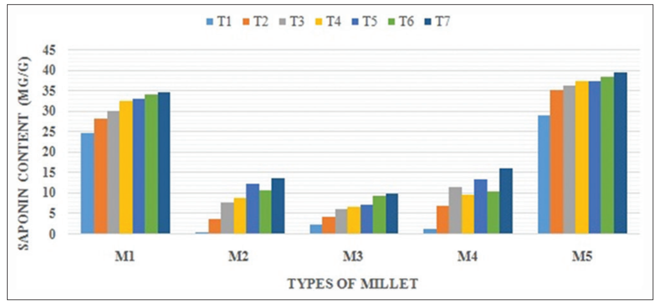

Figure 2: Influence of soaking and germination time in saponin content of five millets (mg/g)
(T1) Control, (T2) 12hr soaking, (T3) 24 hr soaking, (T4) 12hr soaking and 12hr germination, (T5) 24hr soaking and 12hr germination, (T6) 12hr soaking and 24hr germination, (T7) 24hr soaking and 24hr germination , (M1) Finger millet, (M2) Foxtail millet (M3) Kodo millet, (M4) Little millet and (M5) Pearl millet.

treatment with the highest amount of saponin content and pearl millet has a high amount of saponin among other millets

DETERMINATION OF TANNIN

The tannin content recorded from all the millets is presented in Table 2. The tannin content was low in little millet ranged between 1.223±0.021 mg/g in T1 to 0.347±0.045 mg/g in T7. The reduction in tannin was about 50% in little millet. This reduction is mainly due to leaching [26]. In general, tannin affects the digestive tract and its metabolism also toxic which may be recovered through the process of germination which reduces the tannin level in millet flours and indirectly reduces the negative effect of tannin [27]. Tannin content reduces when there is an increase in the germination period.The highest range of tannin was found in finger millet (ranging from 2.077±0.068 mg/g to 0.553±0.050 mg/g) followed by foxtail millet, pearl millet, and Kodomillet.Among treatments, T7 and T5 are found to have the lowest amount of tannin content in all the millets. So, from this, we say that 24h soaking with a difference of germination period influences the tannin content. A similar study was observed in which the tannin is reduced to 50% [28]. A decrease in anti-nutritional factors during germination could lead to leaching of polyphenols in the soaking water [29] and increased enzymatic treatment during germination [30]. In comparison with all treated millets, we found that M4T7 (0.347±0.045 mg/g) has the lowest amount of tannin and M1T1 (2.077±0.068 mg/g) has the highest amount.

PHYTASE ASSAY

Phytase activity of both raw and sprouted millets are shown in Figure 3. Phytase activity was found to be high in Kodo millet ranging from 54.413±0.454 unit/kg in T1 to 60.627±0.335 unit/kg in T7, which is followed by little millet, finger millet, foxtail millet, and pearl millet.

Germination showed a significant increase in the phytase activity of all the samples studied. This may be due to the de nova synthesis of the enzyme at the time of germination [31]. The lowest level of phytase was observed in pearl millet ranging

Table 2: Influence of soaking and germination time on the tannin content of the selected millets

Treatment	Tannin content (mg/g)				
	M1 (1)	M2 (2)	M3 (3)	M4 (4)	M5 (5)
T1	2.077±0.068	1.971±0.038	1.420±0.026	1.223±0.021	1.863±0.107
T2	1.620±0.026	1.830±0.036	1.247±0.045	0.973±0.021	1.360±0.114
T3	1.220±0.035	1.627±0.025	0.930±0.053	0.733±0.040	0.897±0.090
T4	1.473±0.071	1.623±0.021	0.967±0.021	0.740±0.040	0.900±0.079
T5	0.763±0.015	1.433±0.021	0.780±0.010	0.550±0.046	0.733±0.042
T6	1.253±0.015	1.410±0.000	0.753±0.031	0.533±0.031	0.783±0.025
T7	0.553±0.050	1.233±0.042	0.527±0.031	0.347±0.045	0.537±0.032

(1) Finger millet; (2) Foxtail millet; (3) Kodo millet; (4) Little millet; (5) Pearl millet

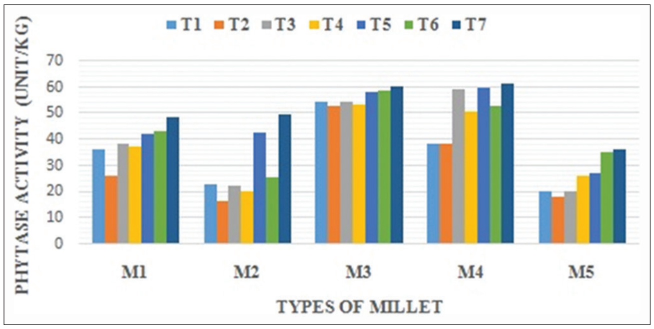

Figure 3: Influence of soaking and germination time on phytase activity (unit/kg)
(T1) Control, (T2) 12hr soaking, (T3) 24 hr soaking, (T4) 12hr soaking and 12hr germination, (T5) 24hr soaking and 12hr germination, (T6) 12hr soaking and 24hr germination, (T7) 24hr soaking and 24hr germination, (M1) Finger millet, (M2) Foxtail millet (M3)Kodo millet, (M4) Little millet and (M5) Pearl millet.

from 20.420±0.365 u/kg in T1 to 36.430±0.373 u/kg. Phytase activity was highest on T7 and T5 respectively due to prolonged soaking. Whereas the phytate level decreases with an increase in germination due to the hydrolysis of phytate to phosphate and myoinositol phosphate [27]. In our study, we found that germination after soaking increases the level of phytase activity in all treated millets. From this study, it is clear that the bounded inorganic phosphorous has been liberated in a high amount during soaking and germination. Results showed that the increased level of phytase activity was accompanied by decreased antinutrient, phytate, and slightly increase phosphorous. Thus the increase in phytase activity and decrease in phytate content are expected to improve phosphorous availability and utilization which would reduce the phosphorous pollution [32].

From this study, we observed that ash content has several variations in which 12h soaking 24h germinated millets showed loss of minerals by low ash content, whereas 24h soaking 24h germinated millets havea considerably high level of ash content, which is due to a decreased level of crude fat and carbohydrate. Moisture content increases with an increase in germination. The highest moisture content was recorded in finger millet when compare to other millets studied. Marked level of increase in moisture content was noticed at the time of germination which may be directly proportional to the fact that the grain absorbs moisture from the soaking medium for its metabolism and reflects on the grain structure. As the time of soaking increases, more number of cells in the seeds are hydrated.

The differential period of soaking and germination resulted in a change of saponin content. The level of saponin was found to be high in the T7 of pearl millet when compared with others. Significantly much difference was not observed among 24 h soaking and 24 h germination period for tannin content. Upon soaking and germination the phytase activity was found to increase when compared with control. Kodo millet was found to have a high amount of phytase activity. The raw and sprouted millets of foxtail have reported being high, the highest flavonoid content was observed in T7 and lowest in pearl millet. DPPH assay was carried out, in which foxtail millet is found to have a high amount of antioxidant activity and pearl millet has a low amount.

FUNDING

The financial support by DST-CURIE for the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore is gratefully acknowledged.

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