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Papori Bora
Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University, Jorhat, Assam, India

Pranati Das
Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University Jorhat, Assam, India

Ruma Bhattacharyya
Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University, Jorhat, Assam, India

Ananta Saikia
Department of Food Science and Technology, Assam Agricultural University, Jorhat, Assam, India

Correspondence
Papori Bora
Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University, Jorhat, Assam, India

Effect of processing on the phytochemical content and antioxidant capacity of proso millet (*Panicum miliaceum* L.) milled fractions

Papori Bora, Pranati Das, Ruma Bhattacharyya and Ananta Saikia

Abstract
The purpose of this study was to promote utility of proso millet by the use of milling. Proso millet grains obtained from the farmer's field of Gosaigaon, Kokrajhar district of Assam were milled to obtain dehusked and polished grains. The flours from these milled fractions along with whole grain flour were evaluated for total phenol, flavonoid, phytate and total antioxidant capacity. A considerable variation in the phytochemical content and antioxidant capacity was found among the milling fractions. The total phenolic and flavonoid content were found to be highest in whole and dehusked grain flours than polished grain flours while phytate content was highest in dehusked (682.50 mg/100g) grain flours than whole (574.74 mg/100g) and polished (194.00 mg/100g) grain flours. Whole grain flour possessed significantly highest total antioxidant capacity (281.79 mg TE/100g) than dehusked (156.93 mg TE/100g) and polished (144.94 mg TE/100g) flours. Depending on technological or nutritional demands, appropriate milled fractions may be chosen based on these results to achieve the desired product.

Keywords: Proso millet, phytochemicals, phenolics, flavonoids, phytate, antioxidant

Introduction
Millets are very hardy crops and can be grown successfully in infertile lands, and in adverse weather conditions like limited rainfall and drought. They are the staple food of the millions of people inhabiting in the arid and semi-arid tropics of the world, and are distributed in most of the Asian and African countries and parts of Europe (Lu *et al.*, 2009) [1]. Millets have substantive potential in broadening the genetic diversity in the food basket and ensuring improved food and nutrition security (Mal *et al.*, 2010) [2] as they are nutritionally comparable or superior to major cereals with respect to energy, protein, vitamins, and minerals (Sehgal and Kwatra 2003) [3] and are the rich source of minerals, nutraceuticals, and higher dietary fibers than rice or wheat (Hadimani and Malleshi 1995) [4]. Therefore, millets can be a promising alternative in solving the problems of food insecurity and malnutrition.

In addition to the various micro and macro nutrients in millets, it contains abundance of bioactive phytochemicals, particularly phenolic compounds, flavonoids and phytic acids (phytate); which act as antioxidant and play potential roles in body's immune system defence (Devi *et al.*, 2014 and Shahidi and Chandrasekara, 2013) [5, 6]. An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates and inhibit other oxidation reactions thereby reducing stress responsible for many degenerative disorders (Deepak *et al.*, 2014) [7]. Studies have shown that antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities (Sala *et al.*, 2002) [8]. Phytochemicals in millets have gained increased interest due to their antioxidant activity, cholesterol lowering properties and prevention and delay of the occurrence of non-communicable diseases (Chandrasekara and Shahidi 2011) [9]. Millet phenolics are receiving growing interest due to their potential role as protective factors against free radical mediated pathologies, such as cancer and atherosclerosis in humans. They also retard oxidative degradation of lipids and thus improve quality and nutritional value of food (Rice-Evans *et al.*, 1996, Kumpulainen and Salonen, 1999) [10, 11].

Proso millet (*Panicum miliaceum* L.) is one of the important minor millets grown extensively in the tropics and a staple food for the low income groups in some countries of the world.

It has excellent nutritional properties with rich source of protein, vitamins, minerals and phytochemicals such as phenolic acids, flavonoids, and phytate with potent antioxidant capacity (Thatola *et al.,* 2011, Mishra, 2012) [12, 13]. It is desirable for human food because it is easily digestible and is gluten-free **(**Reddy *et al*., 2007) [14].

A few varieties of millets including proso millet is commonly cultivated minor millet in Assam especially in lower Assam and adjoining North-Eastern states but it has not been analyzed for its phytochemical profiles and antioxidant potential. Therefore, considering the limited information available in the literature on the phytochemical content and antioxidant properties of proso millet, this study was carried out to investigate the effect of milling on phytochemical content (total phenol, flavonoid and phytate) and antioxidant properties (DPPH free radical scavenging assay) of proso millet.

Materials and Methods

Collection of Sample

Proso millet grains were procured from the farmer's field of Gosaigaon, Kokrajhar district of Assam for the study.

Processing of Sample

Sample was cleaned thoroughly to remove all foreign matters, broken and immature grains. A known amount of cleaned sample was dehusked in a *Satake grain dehusker*, debranned in a *Satake grain polisher* for 35 seconds to obtain the different milled fractions i.e. dehusked grain (unpolished & bran rich fraction) and debranned or polished grain. The whole grain, dehusked grain and the polished grain were then separately ground to a fine flour to pass through B.S. 60 mesh sieve employing an electrical grinder to obtain whole flour, dehusked flour (bran rich fraction) and polished flour (refined flour) respectively. The grinding operation was conducted below 40°C. These milling fractions were stored at 4°C in air tight containers and used for analysis.

Determination of phytochemical content of the samples

Extraction of bioactive compounds from sample

Five grams of dried powdered sample was taken in a 100 ml conical flask and 15 ml of 80% methanol acidified to pH 2.0 with 6N hydrochloric acid was added into it. The extraction step (15ml+15ml+15ml) was done thrice each for 30 minutes in a mechanical shaking machine. Supernatant was filtered using whatman No. 1 filter paper after centrifuging the suspension at 6000 rpm for 15 minutes. Volume was made up to 50 ml with the solvent (80% methanol). The sample was transferred to micro centrifuge tubes and stored at -20°C until analysis.

Determination of Total Phenolic Content (TPC)

Total phenolic content of the extract were determined spectrophotometrically using the Folin-Ciocalteu colorimetric method as described by Bray and Thorpe, 1954 [15] with modifications. Known aliquot (1 ml) of sample extract was taken and oxidized with 1 ml of Folin-Ciocalteu reagent, and the reaction was neutralized with 7.5% sodium carbonate. The resulting blue colour was reconstituted with methanol to a final volume of 10 ml. Incubated at 37˚C for 60 minutes and absorbance was measured at 750 nm in a spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalent/100 g dry weight (mg GAE/100 g DW).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content in the extract was determined by aluminium chloride colorimetric method (Dewanto *et al*., 2002) [16]. The extract (1 ml) was diluted with 4 ml of distilled water in a10 ml volumetric flask. Initially, 0.3 ml of 15% sodium nitrite (NaNO2) solution was added to the volumetric flask, after 5 minutes, 0.3 ml of 10% aluminium chloride (AlCl3) was added; and after 6 minutes, 2 ml of 1.0M sodium hydroxide (NaOH) was added to the reaction mixture and mixed thoroughly. The Absorbance at 510 nm was measured after 30 minutes. The result was expressed as mg of rutin equivalents/100 g dry weight (mg RE/100 g DW).

Determination of phytic acid (phytate) content

Phytate content was determined by the method of Wheeler and Ferrel, 1971 [17]. The absorbance at 480 nm was measured and the result was expressed as mg/g dry weight (mg/g DW). Samples were accurately weight (5g) and transferred into 100 ml conical flasks. A total of 40-50 ml of 3% trichloroacetic acid (TCA) was added and shaken vigorously for 45 minutes on a mechanical shaker. Suspension was centrifuged and 10 ml aliquot of the supernatant was transferred to a 40 ml conical centrifuge tube. Four ml of $FeCl_3$ solution was added to the aliquot by blowing rapidly from the pipette. The content was then heated in a boiling water bath for 45 minutes. Centrifuged (10 to 15 minutes) and the clear supernatant were decanted carefully. The precipitate was then washed twice by dispersing well in 20 to 25 ml 3% TCA, heated in boiling water bath for 5 to 10 minutes and centrifuged. Repeated washing with water. The precipitate was dispersed in a few ml of water and 3 ml of 1.5 N NaOH was added with mixing. Volume was brought to approx. 30 ml with water and heated in boiling water for 30 minutes and filtered hot through a moderately retentive paper whatman 2. The precipitate was washed with 60-70 ml hot water and discarded the filtrate. Dissolved the precipitate from the paper with 40ml hot 3.2 N HNO_3 into a 100 ml volumetric flasks. Paper was washed with several portions of water, collecting the washings in the same flasks. Cooled flasks and contents to room temperature and diluted to volume with water. A 5 ml of aliquot was transferred to another 100 ml volumetric flask and diluted to approx. 70 ml. Twenty ml of 1.5 M KSCN was added and volume was made up, and colour was read immediately (within 1 minute) at 480 nm. A reagent blank was run with each set of sample.

Calculation

$$\text{Phytate P (mg/100g)} = \frac{\mu\text{g Fe x 15}}{\text{Weight of the sample (g)}} \text{ x 100}$$

Determination of total antioxidant capacity (TAC)

The antioxidant activity was determined spectrophotometrically by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Vani *et al.,* 1997) [18]. Different sample aliquots were taken and volume was made up to 1 ml with methanol. 3 ml of DPPH reagent was added to the sample and mixed the sample properly and incubated for 20 minutes at 37°C. Absorbance of the resulting oxidizing solution was measured at 517 nm against methanol as blank. A control was prepared by taking 1.0 ml of methanol as such and treated in the same way as sample.

Calculation

$$\text{DPPH inhibition (\%)} = \frac{(Ac - Ae)}{Ac} \times 100$$

Where, Ac = Absorbance of control
Ae = Absorbance of extract
Total antioxidant capacity (mg TE/100g) = Std. Conc./ Std. % inhibition x Sample % inhibition /Aliquot taken x Volume made up/ Sample taken x 100/ 1000 x Dilution factor

Statistical Analysis

The data obtained was subjected to statistical analysis using 'completely randomized design' with 5 replications to determine differences between treatment means by using Microsoft excel (2007). The significance difference of the treatment values was tested by F-test at 0.05% probability level. Values are means of five (5) replications ± standard deviation. Means with different superscript within the same row are significantly different at $p \leq 0.05$.

Results and Discussion

Phytochemical content of proso millet milled frations

Millet grains have an abundance of phytochemicals, particularly phenolic compounds and phytic acids (phytate). They act as antioxidant and could impart beneficial health effects to prevent and delay the occurrence of non-communicable diseases (NCDs) (Shahidi and Chandrasekara, 2013) [6]. Phytochemical content of proso millet milled fractions is presented in Table 1.

Table 1: Phytochemical content of proso millet milled fractions (per 100 g)

Parameters	Whole	Dehusked	Polished	CD $_{0.05}$
Total phenolic content (TPC) (mg GAE/100g)	164.46±1.89^{a}	145.81±0.94^{b}	69.37±0.86^{c}	1.82
Total flavonoid content (TFC) (mg RE/100g)	133.03±1.87^{a}	119±1.93^{b}	51.23±1.10^{c}	1.43
Phytate (mg)	574.74±12.92^{b}	682.50±12.99^{a}	194.00±5.48^{c}	15.22

Values are mean ± SD of 5 replications; Means with different superscript within the same row are significantly different at $p \leq 0.05$

Total phenolic and total flavonoid content

The perusal of data (Table 1) reveals that total phenolic content of proso millet were 164.46±1.89 mg GAE/100g in whole grain, 145.81±0.94 mg GAE/100g in dehusked grain and 69.37±0.86 mg GAE/100g in polished grain. It is evident from the present study that processing had significant ($p \leq 0.05$) impact on total phenolic content, therefore whole grain possessed highest amount of total phenolics as compared to dehusked and polished grains. Similar to TPC, whole grain flours (133.03±1.87 mg RE/100g) contain more TFC than the milled fractions. Furthermore, TFC also decrease markedly after dehulling and debranning ($p \leq 0.05$, Table 1). The results are in accordance with the observations of Chandrasekara *et al.,* 2012 [19] showed that dehulling decreased the total phytochemical content of whole grain millets and this change was due to the removal of the outer layers of the grain and Suma and Urooj, 2011 [20] who reported that hulls had higher total phenolic and flavonoid content than those of dehulled grains in proso, kodo, foxtail, pearl, little and finger millets, which are similar to the findings of the present study. Earlier study also suggested that phenolics are chiefly present in the husk and outer layers of the kernel. The decrease in phenolic content is progressive as successive dehulling and debranning progressed through the aleurone layer of bran and outer layer of kernel (Devisetti *et al.,* 2014) [21]. This result is consistent with the present study where a gradual decrease of total phenolic and flavonoid content was observed in dehusked and polished grains. Therefore, the results of the present study give further confirmation of the location of most phenol compounds in the outer layers of the grain similar to wheat as previously reported by Beta *et al.,* 2005 [22]. Therefore, the distribution of phenolic compounds in milled fractions of millets may have important implications in end-use applications and in generating health benefits as functional foods.

Phytate Content

Phytate (myoinositol hexa-phosphoric acid) is the major phosphorus storage compound of most seeds and cereal grains, it may account for more than 70 per cent of the total phosphorus. It has been reported that the phytic acid phosphorus constitutes the major portion of total phosphorus in the millets (Ravindran, 1991) [23]. The phytate content of the proso millet is presented in the Table 1. It can be seen that the phytate content of proso millet was 574.74±12.92 mg/100g in whole grain, 682.50±12.99 mg/100g in dehusked grain and 194.00±5.48 mg/100g in polished grain. Significant differences ($p \leq 0.05$) have been observed in phytate content among whole, dehusked and polished grains. Highest amount (682.50±12.99 mg/100g) of phytate in dehusked grain indicated that it is primarily concentrated in the bran layers. These findings are in conformity with those of Suma and Urooj, 2011 [20] in pearl millet and Devisetti *et al.,* 2014 [21] in proso and foxtail millet, where phytate is mostly concentrated in bran layer. A study conducted by Lorenz *et al*., 1980 [24] on 24 samples of proso millet found that phytate content of whole grain proso millets ranged from 170 to 610 mg/100g. Values reported for proso millet in the present study were also within this range. Whereas, the results of the present study found to be higher than the study reported by Thilagavathi *et al*., 2015 [25] that phytate content was maximum in kodo millet (35.17 mg/100g) followed by pearl millet (33.42 mg/100g), proso millet (27.17 mg/100g) and little millet (24.42 mg/100g), respectively. The proso millet phytate of the earlier study by Thilagavathi *et al*., 2015 [25] was much lower than the phytate content of the present study, which may be due to varietal difference and differences in degree of milling. Roopa *et al.,* 2013 [26] observed the significant differences in the phytate content of two local little millet genotype as 115.13 mg/100g and 94.36 mg/100g, respectively These differences in the phytate contents in millet depend on a number of factors such as variety, part of the grain, climatic conditions and cultivation practices etc. (Shahidi and Naczk, 2004) [27].

Antioxidant capacity of proso millet milled fractions

Millets are good sources of natural antioxidants such as polyphenolic compounds, vitamins and other secondary metabolites for the human diet, which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases in addition to other health benefits (Krishaniah *et al*., 2007) [28].

Table 2: Antioxidant capacity of proso millet milled fractions

Millets	DPPH % inhibition	Total antioxidant capacity (TAC) (mg TE/100g)
Whole	31.55±0.09	281.79±0.81[a]
Dehusked	17.57±0.08	156.93±0.69[b]
Polished	16.27±0.31	144.94±2.84[c]
$CD_{0.05}$	-	2.41

Values are mean ± SD of 5 replications; Means with different superscript within the same column are significantly different at $p \leq 0.05$

The values for total antioxidant capacity for proso millet sample by DPPH (2, 2 diphenyl-1-picryl-hydrazyl) radical scavenging assay are presented in Table 2. In the present study, percent (%) inhibition of DPPH by the samples were 31.55±0.09% in whole, 17.57±0.08% in dehusked and 16.27±0.31% in polished proso millet grains. Whole proso millet had the highest total antioxidant capacity (281.79±0.81 mg TE/100g) followed by dehusked flour (156.93±0.69 mg TE/100g), whereas polished proso millet had the lowest value (144.94± 2.84 mg TE/100g). The findings of the present study are comparable with the study reported by Kundgol *et al*., 2014 [29] who analyzed the impact of processing on antioxidant content and antioxidant activity of 10 samples of little millet using DPPH (2, 2-diphenyl- 1-picrylhydrazyl) and found that all the decorticated grain, bran and whole millet grain exhibited antioxidant activity and revealed that the removal of bran drastically reduces the antioxidant activity. Therefore, dehulled grains, as well as the hull fraction of millets, may serve as potential sources of nutraceutical and functional food ingredients in health promotion (Chandrasekara and Shahidi, 2011) [30]. Asharani *et al*., 2012[31] showed that the total antioxidant capacity in finger, little, foxtail and proso millet were 15.3± 3.5, 4.7±1.8, 5.0± 0.09, and 5.1± 1.0 mg TE/g, respectively, which are comparatively lower than the results of present findings.

Conclusion

The results of this study indicated that processing significantly affected the phytochemical content and antioxidant activity of proso millet. Dehulling and subsequent removal of bran (polishing) of proso millet results in variations in the content of phytochemicals and antioxidant activity in whole, dehusked and polished grain flours because these are unevenly distributed in the grain. Phytochemicals are mostly concentrated in the husk and bran fractions and might be easily removed by dehulling and polishing. Milling of proso millet to obtain different fractions may have several advantages. It may lead to the concentration of some interesting components in certain milling fractions (phenolics, flavonoid and phytic acid) which have potent antioxidant capacity. These favourable antioxidant properties of different milled fractions of proso millet flours could be exploited for the development of desired end-use food products. The gluten-free flours from this underutilized millet grain and its milled fractions may also be very attractive for producing composite flours as partial substitutes of wheat in bakery products, snacks, confectionery and other traditional food products.

References

1. Lu H, Zhang J, Liu K, Wu N, Li Y, Zhou K. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. Proceedings of National Academy of Science, 2009, 7367-7372.
2. Mal B, Padulosi S, Ravi SB. Minor millets in South Asia: learnings from IFAD-NUS Project in India and Nepal. M.S. Swaminathan Research Foundation. 2010; 32(2):1-185.
3. Sehgal A, Kwatra A. Processing and utilization of pearl millet for Nutrition Security. Proceeding of national seminar on Recent Trend in Millet Processing and Utilization held at CCS HAU, Hissar, India, 2003, 16.
4. Hadimani NA, Malleshi NG. Studies on milling, physico-chemical properties, nutrient composition and dietary fiber content of millets. Journal of Food Science and Technology. 1993; 30:17-20.
5. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. Journal of Food Science and Technology. 2014; 52(6):1021-1040.
6. Shahidi F, Chandrasekara A. Millet grain phenolics and their role in disease risk reduction and health promotion: A review. Journal of Functional Foods. 2013; 5:570-581.
7. Deepak S, Pawar A, Shinde P. Study of antioxidant and antimicrobial activities of *Andrographis paniculata*. Asian Journal of Plant Science and Research. 2014; 4(2):31-41.
8. Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G *et al*. Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. Journal Pharmacy and Pharmacology. 2002; 54(3):365-371.
9. Chandrasekara A, Shahidi F. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. Journal of Functional Foods. 2011; 3:144-158.
10. Rice-Evans CA, Millar NJ, Bolwell PG, Bramley PM, Pridham JB. Therelative antioxidant activities of plant-derived polyphenolic Flavonoids. Free Radical Research. 1996; 22:375-383.
11. Kumpulainen JT, Salonen JT. Natural antioxidants and anti-carcinogens in nutrition, health and disease. The Royal Society of Chemistry, UK, 1999, 178-187.
12. Reddy Belum VS, Sharma HC, Thakur RP, Ramesh S, Kumar AA. Characterization of ICRISAT-Bred sorghum hybrid parents. ISMN. 2007; 48:1-123.
13. Thathola A, Srivastava S, Singh G. Effect of foxtail (*Setaria italica*) supplementation on serum glucose, serum lipids and glycosylated haemoglobin in type 2 diabetics. Diabetologia Croatica, 2011, 40-41.
14. Mishra N. Development of functional biscuits from soy flour and rice bran. International. Journal of Agricultural and Food Science. 2012; 2:14-20.
15. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Methods Biochemical Analysis. 1954; 1:22-27.
16. Dewanto V, Wu X, Adom KK, Liu RH. Thermal Processing Enhances the Nutritional Value of Tomatoes by increasing total Antioxidant Activity. Journal of Agricultural and Food Chemistry. 2002; 50:3010-3014.

17. Wheeler EL, Ferrel PE. A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry. 1971; 48:312-320.
18. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation, Triphala and its constituents. International Journal of Pharmacology. 1997; 35:313-317.
19. Chandrasekara A, Naczk M, Shahidi F. Effect of processing on the antioxidant activity of millet grains. Food Chemistry. 2012; 133:1-9.
20. Suma PF, Urooj A. Nutrients, antinutrients & bioaccessible mineral content (*in vitro*) of pearl millet as influenced by milling. Journal of Food Science and Technology. 2011; 51:756-761.
21. Devisetti R, Yadahally SN, Bhattacharya S. Nutrients and antinutrients in foxtail and proso millet milled fractions. Evaluation of their flour functionality. Food Science and Technology. 2014; 59:889-895.
22. Beta T, Nam S, Dexter JE, Sapirstein HD. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chemistry. 2005; 82:390-393.
23. Ravindran G. Studies on millets: proximate composition, mineral composition, and phytate and oxalate contents. Food Chemistry. 1991; 39(3):99-107.
24. Loreng K, Dilsaver W. Proso millet, milling characteristics, proximate compositions, nutritive value of flours. Cereal Chemistry. 1980; 57(1):16-20.
25. Thilagavathi T, Banumathi P, Kanchana S, Ilamaran M. Effect of heat moisture treatment on functional and phytochemical properties of native and modified millet flours. Plant Archives. 2015; 15(1):15-21.
26. Roopa U, Kasturiba B, Naik R, Malagi U, Shanthakumar G, Hemalatha S *et al*. Physico-chemical and functional properties of little millet genotypes. Karnataka Journal of Agricultural Science. 2013; 26(4):539-542.
27. Shahidi F, Naczk M. Phenolics in food and nutraceuticals. Boca Raton, FL: CRC Press, 2004, 1-82.
28. Krishnaiah D, Sarbatly R, Bon A. Phytochemical antioxidants for health and medicine-A m ove towards nature. Biotechnology and Molecular Biology Review. 2007; 1(4):097-104.
29. Kundgol NG, Kasturiba B, Math KK, Kamatar MY. Effect of preliminary processing on antiradical properties of little millet landraces. International Journal of farm Sciences. 2014; 4(2):148-154.
30. Chandrasekara A, Shahidi F. Bioactivities and antiradical properties of millet grains and hulls. Journal of Agricultural Food Chemistry. 2011; 59(17):9563-71.
31. Asharani VT, Jayadeep A, Malleshi NG. Natural antioxidants in edible flours of selected small millets. International Journal of Food Properties. 2012; 13(1):41-50.