DOI: 10.21088/ijfnd.2348.9987.161224.2

International Journal of Food, Nutrition and Dietetics
Volume 13 Number 1/January - April 2025

ORIGINAL ARTICLE

Effect of Soaking Time on the Anti-Nutritional Properties of Barnyard Millet (*Echinochloa frumentacea*)

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HOW TO CITE THIS ARTICLE:

Manasa R, Shekhara Naik R. Effect of Soaking Time on the Anti-Nutritional Properties of Barnyard Millet (*Echinochloa frumentacea*). International Journal of Food, Nutrition and Dietetics. 2025;13(1): 11–16.

ABSTRACT

Background: Millets serve as a staple food for a significant portion of the global population. Despite their high nutritional value and therapeutic benefits, they are often overlooked. While millets contain certain anti-nutrients that may have adverse health effects, employing specific processing methods can effectively reduce these compounds.

Materials and methods: In the present study, soaking was used as the method to reduce the anti-nutrients like tannins, phytates and oxalates, phenolics and flavonoids. The grains were washed and soaked for different time periods from 6, 12, 18, 24 hours.

Results: Results showed that Total phenolic content (TPC) content varied from 31.03 to 53.06 mgGAE/100g; Total flavonoid content (TFC) content varied from 120.02 to 173.12 mgCE/100g; Tannin content varied from 2.01 to 3.11 mgTAE/100g; Phytic acid content varied from 2.11 to 3.21 mg/100g; Oxalate content varied from 0.03 to 0.05 µg/100g.

Conclusion: The results indicate that the anti-nutrients of the barnyard millet can be reduced by soaking the millet grains before cooking which also improves the cooking quality and increases the digestibility of the barnyard millet.

KEYWORDS

• Barnyard millet • Soaking • Phytate • Tannin • Oxalate.

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Received: 28-02-2025 **Accepted:** 27-03-2025

INTRODUCTION

Barnyard millet, also known as Japanese barnyard millet, Ooda, Oadalu, Sawan, and Sanwank, is a small-sized grain recognized for its exceptional nutritional value. It is predominantly cultivated in India, China, Japan, and Korea, serving both as a food source for humans and as a fodder.[1] The crop is especially prized for its ability to withstand drought conditions.[2] This millet is rich in essential nutrients, including carbohydrates, proteins, fats, and crude fiber, with notable amounts of calcium and iron. It is easily digestible and contains carbohydrates that are digested slowly, providing a sustained energy release.[3] Due to its nutritional benefits, barnyard millet is an excellent ingredient for a variety of food products, such as baby food, snacks, and dietary foods.[4]

Soaking is a common household method that enhances the nutritional quality of millet grain and flour and reduces the levels of anti-nutrients present in the millet.[5] This process lowers phytic acid content, which can vary depending on the grain species, pH conditions, and the duration of soaking. For instance, significant reductions in phytate levels have been observed during the soaking of soybeans.[6] Studies have investigated how soaking affects the hydration characteristics of finger millet[6-7] and have also explored the effects of soaking in combination with other processing methods like germination, malting, and popping.[7] However, there is limited information on how soaking as pre-treatment, influences the nutritional composition, anti-nutritional factors, phytochemicals, and antioxidant properties of Barnyard millet.

OBJECTIVES

- To evaluate the impact of Soaking on Anti-nutritional properties of barnyard millet.
- To estimate the anti-nutrient composition of barnyard millet with varying soaking time.

MATERIALS AND METHODS

Raw materials: Barnyard millet (*Echinochloa frumentacea*) grains were procured from the local market of Mysuru, Karnataka. The research was conducted in the Department of Food Science and Nutrition at Yuvaraja's College (Autonomous), University of Mysore, Mysuru, Karnataka.

Method of Soaking: The barnyard millet grains were sorted and cleaned to remove impurities. The grains were washed and soaked for different time periods from 6, 12, 18, 24 hours. The water was drained out from grains; it was washed again and dehydrated at 110°C for 6 hours and then milled to produce fine textured powder and used for the analysis.

Anti-nutritional properties of the soaked Barnyard millet:

Total Phenolic content (TPC): The total phenolic content of the extract was determined using the method described by Singleton *et al.*[8] The procedure involved oxidizing the extract dilutions with 2.5 mL of 10% Folin-Ciocalteu reagent (v/v), followed by neutralization with 2.0 mL of 7.5% sodium carbonate ($Na_2 CO_3$). The reaction mixture was then incubated at 45°C for 40 minutes. After incubation, the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard, and the total phenolic content was calculated in terms of gallic acid equivalents.

Total Flavonoid content (TFC): The total flavonoid content was determined using a colorimetric method. To perform the analysis, 0.075 mL of 5% sodium nitrite ($NaNO_2$) was mixed with 0.5 mL of the sample solution (at a concentration of 1 mg/mL) and allowed to stand for 6 minutes. Subsequently, 0.15 mL of a 10% aluminium chloride ($AlCl_3$) solution was added, and the mixture was left at room temperature (28±2°C) for 5 minutes. After this, 0.5 mL of 1M sodium hydroxide (NaOH) was added, and the total volume was adjusted to 2.5 mL using distilled water. The absorbance of the solution was then measured at 510 nm with colorimeter, using a blank with only the extraction solvent as a reference. The total flavonoid content was determined from standard curve prepared using different concentrations of quercetin standard and expressed as mg quercetin equivalent (QE/100g) of the sampleweight.[9]

Tannin content: The tannin content was measured using the Folin-Denis reagent method, following the procedure described by Makkar.[10] Aliquots of the tannin extract (0.05, 0.2, and 0.5 mL) were transferred into

test tubes and the volume was adjusted to 1.0 mL with distilled water. Next, 2.5 mL of sodium carbonate reagent was added to each tube. The test tubes were shaken thoroughly, and the absorbance was recorded at 725 nm after 40 minutes. The tannin content was then calculated as tannic acid equivalents based on the standard curve.

Phytic Acid content: A 100 µL sample was diluted with distilled water to a final volume of 1.4 mL, and 1.0 mL of ferric ammonium sulfate solution (50µg/mL) was added. After mixing, the test tubes were sealed and heated in a boiling water bath for 20 minutes. Once cooled to room temperature, 5 mL of amyl alcohol was added to each tube, followed by 1 mL of ammonium thiocyanate solution (100g/L). The tubes were then centrifuged, and the color intensity of the amyl alcohol layer was measured at 465 nm using a spectrophotometer, with an amyl alcohol blank used as reference, exactly 15 minutes after the addition of ammonium thiocyanate. The total phytate content was determined using a standard curve generated from different concentrations of sodium phytate.[11]

Oxalate Content: A 1g sample was weighed and placed into a 100 ml conical flask. Then, 75 ml of 1.5 M H_2SO_4 was added, and the solution was gently stirred intermittently using a magnetic stirrer for approximately 1 hour. Afterward, the mixture was filtered through Whatman No. 1 filter paper. A 25 ml portion of the filtrate (sample extract) was collected and titrated while hot (80-90°C) with a 0.05 M $KMnO_4$ solution until a faint pink color appeared and persisted for at least 30 seconds. The volume of $KMnO_4$ solution used was then recorded.[12]

Statistical analysis: The anti-nutritional properties of soaked barnyard millet was performed in triplicates and the mean values were computed by applying Holm-Sidak method of statistical analysis where, the obtained experimental values are mean ± SD (n=3) *p value < 0.05.[13]

Results and Discussion: The barnyard millet was soaked for 0-24 hours and the results for anti-nutritional properties were summarized in figure1 and 2 and discussed below.

Total Phenolic content (TPC): TPC content varied from 31.03 to 53.06 mg GAE/100g (Fig 1); as compared to the un-soaked millet, there was a 41.51% decrease in 24 hrs soaked barnyard millet. This result agrees with the previous findings that total phenols decrease with processing.[14-15] Hithamani and Srinivasan (2014), said during soaking of pearl millet and finger millet, the polyphenolic content was reduced due to activation of the enzyme polyphenol oxidase.[16] Alka and Kapoor (1996) found a 39.57% reduction in polyphenol content in pearl millet.[17] Another study by Ajay et al., (2017) found a 70% reduction in polyphenols in finger milletwhen soaked for 24 hrs.[18] Abioye *et al.,* (2022) found that total phenolic content of the finger millet decreased from 40.29 to 16.00 mg/100 g when soaked for 24 hrs.[19]

Total Flavonoid content (TFC): TFC content varied from 120.02 to 173.12 mgCE/100 g (Fig 2); there was a decrease in the TFC contentas the soaking time increased. As compared to the un-soaked millet, there was a 30.67% decrease in 24 hrs soaked barnyard millet. This is in contrast with Abioye *et al.,* (2022), who found that the flavonoid content of the finger millet increased from 66.36 to 103.9 mg/100 g when soaked for 24 hrs.[19]

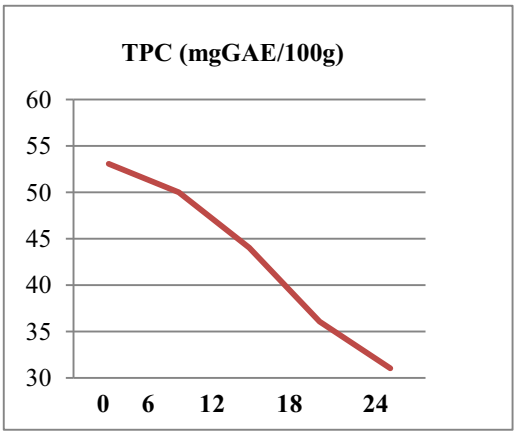

Fig 1: Total Phenolic Content (TPC)

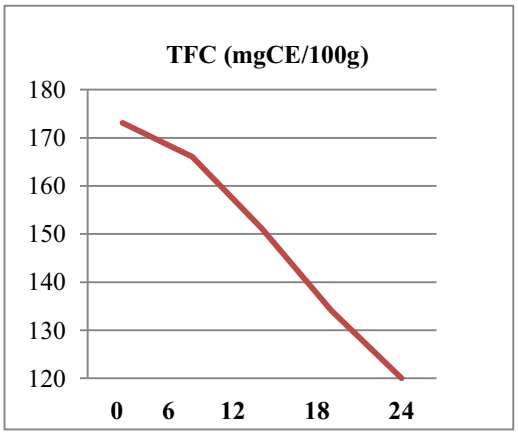

Fig 2: Total Flavonoid Content (TFC)

Tannin content: Tannin content varied from 2.01 to 3.11 mg TAE/100g (Fig 3); as the soaking time increased there was a decrease in the tannin content. As compared to the un-soaked millet, there was a 35.36% decrease in 24hrs soaked barnyard millet. This is in line with Hithamani and Srinivasan (2014), showed a reduction in tannin content from 2.72 to 0.70 mg/g after soaking of pearl millet and finger millet.[16] According to Abioye *et al.*, (2022), tannin contents decreased from 2.23 to 1.25 mg/100 g when finger millet was soaked for 24 hrs.[19] Bhuvaneshwari, G *et al.*, (2020), showed there was 50% reduction in tannin content when little milletsoaked for 24 hrs.[20]

Phytic acid content: Phytic acid content varied from 2.11 to 3.21 mg/100g (Fig 4); as the soaking time increased there was a decrease in the phytic acid content. As compared to the un-soaked millet, there was a 34.26% decrease in 24 hrs soaked barnyard millet. Because during soaking period there may be leaching of phytate ions into the soaking water under the influence of a concentration of gradient, which governs the rate of diffusion. The study conducted by Alka and Kapoor (1996) found a 36.8% reduction in Phytic acid in pearl millet.[17] Abioye *et al.*, (2022), showed that phytate content reducedfrom 0.75 to 0.38 mg/100g when finger millet was soaked for 24 hrs.[19] Lestienne et al., (2005)also noted that soaking of small millet, maize and sorghum seeds at room temperature for 24hrs decreases the phytic acid content by 50%.[22]

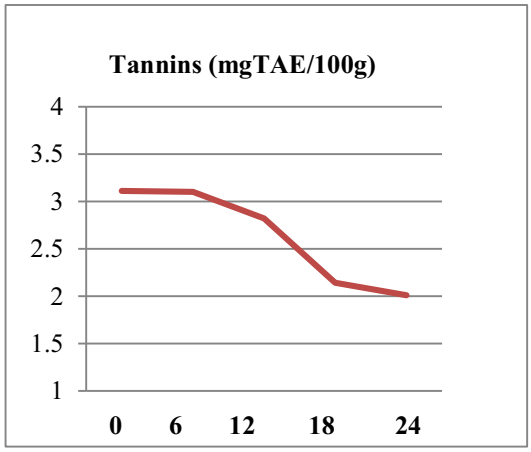

Fig 3: Tannin Content

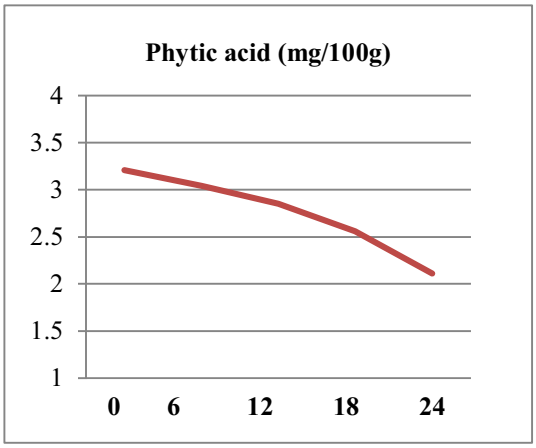

Fig 4: Phytic Acid Content

Oxalate content: Oxalatecontent varied from 0.03 to 0.05 µg/100g (Fig 5); as the soaking time increased there was a decrease in the oxalate content. As compared to the un-soaked millet, there was a 29.94% decrease in 24hrs soaked barnyard millet.Abioye *et al.*, (2022), showed that oxalate content decreased from 1.48 to 0.39 mg/100g when finger millet was soaked for 24hrs.[19]

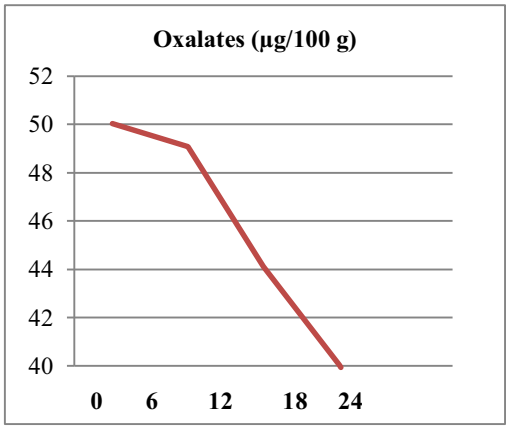

Fig 5: Oxalate Content

Conclusion: Present study showed that ant-nutrients can be reduced by soaking barnyard millet at different time period. When soaked for 24hrs; TPC content was reduced by 41.51%, TFC was reduced by 30.67%, tannins were reduced by 35.36%, phytic acid by 34.26%, and oxalates by 29.94%. There was a highest percentage reduction in TPC and tannins when soaked for 24hrs. Hence, soaking can be used as one of the processing methods to reduce the anti-nutrients in the barnyard millet and to improve its cooking quality and increase its digestibility and also to improve the bioavailability of other nutrients.

Conflict of Interest: None

Funding: None

Ethical Declaration: No ethical issues involved.

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