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Effect of Germination, Drying, Milling and Sieving on the Nutritional Quality of Foxtail Millet (*Setaria italica***)**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed to evaluate the effect of germination, drying, milling and sieving on the protein and phytate content of foxtail millet

Study Design: This was an experimental, laboratory-based study.

Place and Duration of Study: The study was conducted at the Department of Dairy Microbiology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, between January 2024 and October 2024.

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Methodology: Soaked foxtail millet (*Setaria italica*) was germinated at different time-temperature combinations (20, 25 and 30°C for 24, 48 and 72 hours). Germination was optimized based on its effects on reducing phytate content and enhancing protein content. Post-germination, the millet was dried at 20°C, 25°C, and 30°C to a final moisture content of 5–7%, ensuring uniform moisture reduction while preserving nutritional quality. The optimum drying temperature was selected based on its influence on protein and phytate levels. The dried millet was milled into fine flour and sieved through a 150-micron sieve to achieve uniform particle size. The protein content was determined using the micro-Kjeldahl method, while phytate content was estimated via titration with ferric chloride. Statistical analysis was performed using ANOVA, with a critical difference (CD) at a 5% significance level used to determine significance among treatments.

Results: Germination at 30°C for 48 hours resulted in the highest protein content (14.76%) and significantly reduced phytate levels (1.59 mol/kg). Drying at 30°C for 48 hours achieved a moisture content of 6.32%, with protein content increasing to 15.10% and phytate content slightly rising to 1.65 mol/kg. Milling and sieving further reduced both protein (13.23%) and phytate content (1.40 mol/kg), with a statistically significant reduction observed only in phytate levels.

Conclusion: Germination at 30°C for 48 hours significantly enhanced protein content and reduced phytate levels in foxtail millet, while drying and sieving processes further optimized nutritional quality. These findings highlight the potential of tailored processing techniques to improve the functional and nutritional value of millet-based products.

Keywords: Foxtail millet; germination; drying; milling; sieving; antinutritional factors; nutrient bioavailability.

ABBREVIATIONS

FSSAI: Food Safety and Standards Authority of India

1. INTRODUCTION

Millets, particularly foxtail millet (*Setaria italica*), are gaining prominence as nutrient-dense cereals with potential health benefits, offering an array of essential macronutrients, dietary fiber, and micronutrients. These grains also contain antinutrients, such as phytates and protease inhibitors, which limit the bioavailability of nutrients. Addressing these limitations through targeted processing techniques is critical to enhance their nutritional value and functional applications (Santhosh et al., 2024a).

Germination is a widely recognized approach to improving the flavor, digestibility, and nutrient profile of cereals and legumes. By degrading antinutrients such as phytates and protease inhibitors, germination significantly enhances the bioavailability of essential nutrients (Bhuvaneshwari et al*.,* 2020). Research demonstrates that germination increases protein content and micronutrient levels while reducing antinutrient concentrations in millets more effectively than soaking, fermentation, or milling (Singh et al*.,* 2017). For instance, germination enhanced protein content by up to 50% in pearl millet and by 2.5 g/100 g in finger millet, while also elevating micronutrient levels (Chauhan,

2018; Akinola et al., 2017). However, excessive germination durations may result in protein degradation due to protease activity, highlighting the importance of optimizing germination conditions (Kaur and Gill, 2021).

Drying, a critical post-harvest process, influences both the safety and nutritional quality of millet products. Effective drying reduces moisture content, thereby minimizing microbial spoilage while preserving functional properties (Wani et al*.,* 2022). Studies show that optimal drying conditions can enhance nutrient stability and reduce microbial contamination, with advanced methods such as freeze drying and fluidized bed drying significantly improving protein content and reducing phytates (Jayashree et al., 2024; Shingare and Thorat, 2013). Conversely, hightemperature drying methods can lead to nutrient losses, including protein denaturation (Devi et al., 2019).

Milling and sieving further modify the nutritional composition of millets. Milling, which removes the bran and germ, enhances protein digestibility and reduces antinutrient levels, such as phytic acid and tannins (Rathore et al., 2016). Sieving, a complementary step, has been shown to selectively reduce antinutrients concentrated in the bran while altering protein and micronutrient content (Langó et al., 2018). However, these processes may also result in nutrient losses, as observed with decreased protein and mineral levels in sieved and milled millet flours (Devisetti et al., 2014; Ahmed et al*.*, 2016).

This study investigates the cumulative effects of germination, drying, milling, and sieving on the nutritional quality of foxtail millet. By systematically evaluating changes in protein content, and phytate levels, the research aims to optimize these processing methods to enhance the functional and nutritional profile of foxtail millet-based products. The findings are expected to contribute valuable insights for developing nutrient-enriched millet products for dietary and industrial applications.

2. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

Foxtail Millet (*Setaria italica)* was procured from a reputed local market in Bengaluru, Karnataka, India.

2.2 Germination, Drying, Milling and Sieving Procedure

Foxtail millet (Setaria italica) was soaked in potable water at a millet-to-water ratio of 1:2. The soaking process was carried out at 30°C for 24 hours to facilitate hydration and prepare the millet for subsequent germination (Santhosh et al. 2024b).

The soaked millet was drained, tied in sterile muslin cloth, and subjected to germination at three temperatures (20°C, 25°C, and 30°C) for three different durations (24, 48, and 72 hours). Germination conditions were maintained under controlled environments using incubators, and the samples were evaluated to determine the optimum time-temperature combination based on the dual objectives of reducing phytate content and enhancing protein content.

Following germination, the millet was dried at three temperatures (20°C, 25°C, and 30°C) until a final moisture content of 5–7% was achieved. The drying process was monitored to ensure uniform moisture reduction while preserving the desired nutritional properties. The optimum drying temperature was determined based on its impact on reducing phytate content and improving protein content.

The dried millet grains were milled into fine flour and then sieved using a 150-micron sieve to achieve uniform particle size.

Following milling and sieving, the protein and phytate contents of the final millet flour were analyzed. Additionally, at each stage (soaking, germination, and drying), the millet samples were analyzed for protein and phytate levels to determine the most effective process conditions. All analyses were conducted in triplicate to ensure reliability and reproducibility of the results.

2.3 Estimation of Protein Content

The micro-Kjeldahl protein technique was used to determine the protein content. A Kjeldahl tube was filled with 0.5g of thoroughly mixed sample, 0.1ml of 5% copper sulphate solution, 1.5g of potassium sulphate and five to ten boiling aids. Next, 15ml of concentrated sulphuric acid were introduced via the tube's wall. After a gentle mixing process, the contents were digested until they were clear and devoid of any leftover substance. Then, the digest was let to cool to 25±2℃. After adding 100ml of distilled water, it was placed in an auto distillation unit and allowed to distill with 50% sodium hydroxide. The ammonia that was released was then trapped in 25ml of boric acid. 25ml of distillate were titrated with 0.02N hydrochloric acid until the colour turned pink. Then, same procedure was followed for blank by substituting the sample with 1ml of water and 0.17g of sucrose. The total nitrogen and crude protein percentages were then calculated using the formula provided below (FSSAI 03.016:2022)

$$
W_n = 1.4007 \times (V_S - V_B) \times N
$$

W

Crude protein $(\%) = W_n \times 6.25$

Where,

 W_n = Nitrogen content of sample, expressed as % by mass

 V_S = Volume in ml of the standard HCl used for sample

 V_B = Volume in ml of the standard HCl used for blank test

 $N =$ Normality of standard HCl

 $W =$ Mass of test portion in g

2.4 Estimation of Phytate Content

About 4.0g of grounded sample was steeped in 100ml of 2% hydrochloric acid for 3h before being filtered using Whatman filter paper. 25ml of this filtrate and 5ml of 0.3% ammonium thiocyanate solution as an indicator were taken and 53.5ml of distilled water was added after setting it to proper acidity and titrated against standard ferric chloride solution (0.00195g of iron per ml) until brownish yellow colour appeared which persisted for 5 minutes (Sharma et al., 2016).

$$
T \times 564.11
$$
\nPhytate content (mol/kg) = M

Where,

 $T =$ Titre value $M =$ Molar mass of phytate in kg

2.5 Estimation of Moisture Content

5.0g sample was taken and placed in a dish that had been previously dried, weighed along with lid. The dish was then placed in oven at 130±3°C for 2h. After that, the dish was placed in a desiccator with a lid on it and allowed to cool to room temperature (25±3℃). Then it was weighed once again. (FSSAI 03.005:2022).

$$
\frac{W_1-W_2}{W_1-W_2} \times 100
$$

Moisture (%) = $\frac{W_1-W_2}{W_1-W}$

Where,

 $W =$ Mass in g of the empty dish

 W_1 = Mass in g of the dish with the test portion before drying

 W_2 = Mass in g of the dish with the material after drying

2.6 Statistical Analysis

The data was analysed using R software [R. version 4.1.2 copyright] for statistical computing. Data on the response variables was collected for three replications of the trails and the ANOVA tables was prepared to analyse the data. The critical difference was calculated (*P=.05*), where the F value was significant, and used to identify whether significant differences existed and indicated in the table using superscripts.

$$
\frac{\sqrt{2} \times MSS(E) \times \text{ta}}{\text{Critical difference (CD)}} = \frac{\sqrt{2} \times MSS(E) \times \text{ta}}{\text{r}}
$$

Where,

MSS (E) = Mean Sum of squares of the error $r =$ number of replications

tα = table t value of the α level of significance

3. RESULTS AND DISCUSSION

3.1 Effect of Germination Parameters on the Protein and Phytate Content of Foxtail Millet

The influence of different germination timetemperature combinations on the protein and phytate content of foxtail millet was evaluated, as presented in Table 1. The initial sample, which was soaked at 30°C for 24 hours, served as the baseline with a protein content of 10.99% and a phytate content of 1.79mol/kg.

3.1.1 Protein content

Germination significantly influenced the protein content of foxtail millet, with variations observed across different time-temperature combinations.

At 20°C, protein content increased from 13.50% at 24 hours to 14.07% at 48 hours, followed by a decrease to 11.86% at 72 hours. A similar trend was observed at 25°C, where protein content rose from 13.70% at 24 hours to a peak of 14.32% at 48 hours, then dropped to 11.68% at 72 hours. The most substantial improvement occurred at 30°C, where protein content reached 14.76% at 48 hours before declining to 11.43% at 72 hours. These results indicate that 30°C for 48 hours is the optimal germination condition for maximizing protein content.

The decline in protein content beyond 48 hours at all temperatures is likely due to proteolytic activity, as protease enzymes degrade proteins to support seedling development. Kaur and Gill (2021) observed similar trends in wheat, barley, sorghum, and pearl millet, attributing this decline to enzymatic degradation during prolonged germination. Wang et al*.* (2025) also demonstrated that germination enhances amino acid content due to increased enzymatic activity, which hydrolyzes and breaks down the endosperm, as seen in germinated brown millet at 30°C for 24 hours.

The findings of this study are consistent with those of Sharma et al*.* (2018), who reported an increase in protein content from 10.60% to 13.75% in foxtail millet germinated at 25°C for 72 hours. Additionally, Sharma and Niranjan (2018) emphasized the significance of the 25–30°C range for achieving optimal germination outcomes, supporting the conclusion that 30°C is ideal for enhancing protein content in foxtail millet.

Note: CD = Critical Difference and control is foxtail millet soaked at 30C for 24 hours in 1:2 millet-to-water ratio and all the values are average of three trials (n=3) and same superscript indicate non-significance while different, indicate statistically significant difference at P=.05

Fig. 1. Effect of time-temperature combinations of germination on the protein and phytate content of foxtail millet

3.1.2 Phytate content

Phytate content, an antinutritional factor, decreased progressively with increasing germination time, with variations observed across different temperatures. The baseline sample had a phytate content of 1.79 mol/kg.

At 20°C, phytate content declined from 1.75 mol/kg at 24 hours to 1.58 mol/kg at 72 hours. A similar trend was noted at 25°C, where phytate levels decreased from 1.70 mol/kg at 24 hours to 1.51 mol/kg at 72 hours. The most significant reduction was observed at 30°C, where phytate content decreased from 1.63 mol/kg at 24 hours to 1.50 mol/kg at 72 hours.

Higher temperatures and extended germination durations were particularly effective in reducing phytate levels. These findings align with those of Sharma et al. (2018), who reported a reduction in phytic acid levels from 8.89 to 5.42 mg/g in foxtail millet germinated at 25°C for 72 hours. The reductions observed in the present study emphasize the effectiveness of germination as a strategy to mitigate antinutritional factors in foxtail millet.

Overall, germination at 30°C for 48 hours was identified as the optimal condition for achieving maximum protein content and significantly reducing phytate levels. Extending germination beyond 48 hours led to diminishing
returns, particularly for protein content, returns, particularly for protein content, underscoring the importance of balancing germination parameters to achieve desired nutritional outcomes.

3.2 Effect of Drying Parameters on Foxtail Millet

3.2.1 Effect of drying temperature and time on the moisture content of foxtail millet

The impact of various temperature and drying duration combinations on the moisture content of foxtail millet is presented in Table 2. The initial moisture content of the millet was 23.62%, which decreased significantly under all drying conditions tested. The results clearly demonstrate that both drying temperature and duration significantly influence moisture reduction, with higher temperatures and longer durations leading to lower moisture content.

At 20°C, moisture content showed a progressive reduction with increased drying time, decreasing to 16.72% after 24 hours and further to 11.64%, 8.45%, and 6.94% at 48, 72, and 96 hours, respectively. Each increment in drying time resulted in a statistically significant reduction, indicating that prolonged drying at 20°C effectively reduces moisture content.

Drying at 25°C achieved greater reductions in moisture content compared to 20°C. After 24 hours, the moisture content dropped to 15.45%, while 48 hours of drying resulted in 9.54%. Prolonging the duration to 72 hours further reduced the moisture content to 6.87%, underscoring the effectiveness of moderate temperatures combined with extended drying periods.

The most substantial moisture reductions occurred at 30°C. After 24 hours of drying, moisture content significantly decreased to 13.34%. Extending the duration to 48 hours reduced the moisture content to 6.32%, and 72 hours of drying resulted in the lowest observed moisture content of 6.14%. These findings highlight the efficacy of higher drying temperatures in rapidly achieving low moisture levels.

The critical difference (CD) at the 5% significance level was 0.914, confirming that the observed differences in moisture content across various conditions were statistically significant. The results demonstrate that drying at 30°C for 48 or 72 hours was the most efficient in achieving the lowest moisture content, suggesting these parameters are optimal for enhancing the storage stability of foxtail millet.

The reduction in moisture content aligns with previous research. Jayashree et al*.* (2024) observed similar reductions during air drying of fermented millet mixes, where moisture content dropped to as low as 5.74% at 30°C. These results corroborate the effectiveness of higher temperatures, such as 30°C, for moisture removal in foxtail millet. Similarly, Bolaji et al*.* (2014) highlighted the influence of temperature and time on moisture reduction in maize-based products, observing non-linear reductions comparable to the trends seen in this study.

Putra and Ajiwiguna (2017) emphasized that higher air temperatures facilitate rapid moisture evaporation due to increased water vapor absorption capacity. This mechanism supports the substantial reductions observed in the present study, particularly at 30°C. Additionally, Do Nascimento et al. (2015) reported that moisture removal primarily occurs through diffusion, a process that becomes more efficient at higher temperatures, further validating the results of this study.

In terms of microbial safety, Wani et al*.* (2022) noted that moisture levels between 12–14% in millet flours resulted in low microbial counts. The moisture levels achieved in this study, as low as 6.14%, suggest that the tested drying conditions could further inhibit microbial growth, thereby enhancing the safety and shelf life of foxtail millet.

In conclusion, drying at 30°C for 48 or 72 hours is the most effective approach for minimizing moisture content while ensuring product quality and microbial safety. These findings underscore the importance of optimizing drying parameters for the long-term storage and functional quality of foxtail millet.

Note: CD = Critical Difference and control is foxtail millet soaked at 30°C for 24h and germinated at 30°C for 48h before drying and all the values are average of three trials (n=3) and same superscript indicate non-significance while different, indicate statistically significant difference at P=.05

3.2.2 Effect of drying time-temperature combinations on the protein and phytate content of foxtail millet

All the drying conditions resulting in a moisture content less than 7% was selected for further protein and phytate analysis, which is presented in Table 3.

3.2.2.1 Protein content

The protein content of foxtail millet was significantly influenced by the drying temperature and time, as presented in Table 3. Before drying,

the protein content was 14.76%, establishing a baseline. The findings revealed a slight increase in protein content to 14.93% after drying at 20°C for 96 hours, although this change was not statistically significant. Conversely, drying at 25°C for 72 hours reduced the protein content to 14.34%, indicating a statistically significant decrease.

Drying at 30°C yielded the highest protein contents among the tested conditions. After 48 hours at 30°C, the protein content increased to 15.10%, and further drying to 72 hours resulted in the highest protein content of 15.22%. However, the difference between these two conditions was not statistically significant. These results suggest that drying at 30°C effectively enhances protein concentration, likely due to the removal of moisture concentrating the protein fraction within the dry matter.

The observed increase in protein content aligns with the findings of Jayashree et al*.* (2024), who reported a rise in protein content from 7.7% to 11.94% during freeze-drying of a fermented probiotic finger millet mix. Although freeze-drying operates at lower temperatures, the concentration effect from moisture reduction appears consistent across different drying methods. This mechanism is particularly evident in the 30°C drying conditions, where moisture loss likely concentrated the nutrient components, including protein.

3.2.2.2 Phytate content

Phytate content, an antinutritional factor, exhibited an increasing trend with higher drying temperatures and longer drying times. The initial phytate content was 1.59 mol/kg, which increased to 1.62 mol/kg after drying at 20°C for 96 hours, representing a statistically significant rise. Drying at 25°C for 72 hours further elevated the phytate content to 1.63 mol/kg.

At 30°C, the phytate content was the highest among all conditions. After drying at 30°C for 48 hours, the phytate content increased to 1.65 mol/kg, while extending the duration to 72 hours resulted in the highest recorded phytate content of 1.67 mol/kg. The increasing phytate levels with higher drying temperatures suggest a concentration effect like that observed for

protein, as moisture removal concentrates all dry matter components, including antinutritional factors.

These findings are in line with Jayashree et al*.* (2024), who observed an increase in phytate content from 1.32 mol/kg to 1.74 mol/kg during freeze-drying of millet. While drying enhances protein concentration, it also concentrates antinutrients like phytates, potentially affecting the bioavailability of minerals.

3.3 Effect of Milling and Sieving on the Protein and Phytate Content of Foxtail Millet

The sieving of millet flour resulted in reductions in both protein and phytate content compared to the unsieved samples. The protein content decreased from 15.10% to 13.23% after sieving. While this reduction was observed, it was not statistically significant. On the other hand, the phytate content showed a statistically significant decrease from 1.65 to 1.40 mol/kg. These results highlight the impact of sieving on both the nutritional and antinutritional components of millet flour.

The reduction in protein content aligns with findings from other studies that have reported similar effects of particle size reduction and milling processes on grains and legumes. For instance, Ahmed et al*.* (2016) observed a decrease in the protein content of Indian lentil flour from 27.55% to 20.97% after sieving with a 74 μm mesh. This decline is likely attributed to the removal of nutrient-dense outer layers, such as the bran, which are rich in proteins and other essential nutrients. As sieving primarily separates the finer starchy endosperm from the coarser bran, the resulting flour tends to have lower protein concentrations.

Note: CD = Critical Difference and control is foxtail millet soaked at 30°C for 24h and germinated at 30°C for 48h before drying and all the values are average of three trials (n=3) and same superscript indicate non-significance while different, indicate statistically significant difference at P=.05

Fig. 3. Effect of drying temperature and time on the protein and phytate content of foxtail millet

Note: CD = Critical Difference and all the values are average of three trials (n=3) and same superscript indicate nonsignificance while different, indicate statistically significant difference at P=.05

The reduction in phytate content from 1.65 to 1.40 mol/kg observed in this study corroborates the findings of Langó et al*.* (2018), who reported a decrease in phytate levels in sorghum (Albita variety) from 0.65 to 0.45 g/100 g after sieving. Similarly, Devisetti et al*.* (2014) demonstrated that milling reduced phytic acid levels in foxtail millet from 5.4 to 1.9 mg/g and in proso millet from 7.2 to 2.2 mg/g. These studies suggest that phytates, predominantly concentrated in the bran and aleurone layers, are significantly reduced through sieving or milling processes that selectively remove these layers.

Further supporting evidence comes from D'Amico et al*.* (2019), who reported protein losses during the inverse milling of quinoa, with protein levels decreasing from 13.18% to 11.10%. Drakos et al*.* (2017) also documented a minor reduction in protein content in barley flour after jet milling, where protein levels decreased from 9.52% to 9.39%. These trends suggest that while the extent of reduction in protein and phytate content may vary depending on the grain

and milling method used, the general pattern of nutrient loss during sieving or similar processes is consistent.

The observed changes in nutrient composition are attributed to the selective removal of bran and other outer layers of grains, which are rich in proteins and antinutrients such as phytates. The findings further reinforce the idea that phytic acid can serve as a reliable indicator of antinutrient levels concentrated in these layers. As sieving effectively reduces these layers, it provides a practical method for modifying the nutrient and antinutrient profile of flours.

4. CONCLUSION

The present study explored the effects of germination, drying, and sieving on the protein and phytate content of foxtail millet, revealing significant insights into the optimization of these processes for enhanced nutritional quality. Germination emerged as a highly effective method for improving protein content and reducing phytate levels, with the optimal conditions identified as germination at 30°C for 48 hours. Under these conditions, protein content reached a peak of 14.76%, while phytate levels were reduced to 1.59 mol/kg. These results demonstrate the potential of germination to enhance the bioavailability of nutrients and reduce antinutritional factors in foxtail millet, aligning with previous studies on other cereal grains.

Drying parameters were found to significantly influence the nutrient profile of foxtail millet. Drying at 30°C for 48–72 hours effectively reduced the moisture content to levels as low as 6.14%, ensuring extended storage stability while preserving protein content. However, a trade-off was observed with an increase in phytate concentration, which underscores the need for careful control of drying conditions to maintain a balance between nutritional benefits and antinutritional factors. These findings emphasize the critical role of drying in maintaining the functional and storage quality of millet-based products.

The sieving process had notable effects on both protein and phytate content. While protein levels decreased marginally from 15.10% to 13.23% due to the removal of nutrient-rich outer layers, phytate content was significantly reduced to 1.40 mol/kg. This reduction highlights sieving as a valuable step in minimizing antinutritional factors, despite the associated slight decline in protein content. These results demonstrate the need to tailor processing techniques based on the intended nutritional and functional requirements of millet-based products.

Overall, the study provides a comprehensive framework for optimizing germination, drying, and sieving processes to enhance the nutritional quality of foxtail millet. The findings contribute to the growing body of evidence supporting the value of millets as functional foods, particularly for improving protein intake and reducing antinutritional components. Future research should focus on integrating these optimized processes into large-scale production systems and exploring their impact on other bioactive components to maximize the health benefits of millet-based formulations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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