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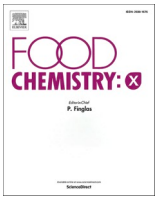

Effects of milling degree on proximate composition, functional components and antioxidant capacity of foxtail millet

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ABSTRACT

The effects of milling degree on proximate compositions, phytic acid (PA), γ-aminobutyric acid (GABA), phenolics and antioxidant capacity of foxtail millet, as well as color characteristics, were investigated. As milling degree increased, the percentage of total starch content increased continuously, while the total protein, crude fat, total carotenoid and PA contents of foxtail millets increased firstly and then decreased. For the whole milling process, the total ash and GABA contents, total phenolic content (TPC) and total flavonoid content (TFC) of foxtail millet decreased with varying degree. The identified 32 individual phenolic compounds were significantly reduced, among which 7 phenolic compositions were undetectable. The antioxidant capacity of foxtail millets also demonstrated a discernible decline. Correlation analysis showed significant positive correlations between b* value and TCC, L* value and starch, TPC and antioxidant capacity. It should be advocated to decrease milling degree for retaining more nutrients and functional components of foxtail millet.

1. Introduction

Foxtail millet, a member of *Poaceae* family of plants, is one of the world's important food crops. As one of the world's oldest crops, foxtail millet has been cultivated for more than 8000 years (Zhang et al., 2017), which is still one of the main crops in arid and subarid regions, mainly grown in Asia and Africa (Sharma & Niranjan, 2017). Foxtail millet grains contain abundant starch, protein, fat, minerals, vitamins and dietary fibers (Xiang et al., 2019). And it is also a good source of natural functional ingredients, such as polyphenols and carotenoids, which assist in reducing chronic and degenerative disorders, such as cardiovascular diseases, hypertension and abnormal cholesterol metabolism (Zhang & Liu, 2015). In addition, foxtail millet also contains quite high content of γ-aminobutyric acid (GABA), which is an important inhibitory neurotransmitter playing a crucial part in the reduction of physical and mental fatigue (Li et al., 2023).

Whole grains are receiving increased attention and strongly recommended in daily diet. And it has been widely recognized that whole grains retain more essential nutrients and phytochemicals, which are beneficial to human health, especially contributing to reducing the occurrence of diverse chronic diseases, such as diabetes, cardiovascular and gastrointestinal tumors, compared with refined grains (Wei et al., 2022). Foxtail millet, as an accredited whole grain resource, is traditionally milled repeatedly and polished to remove the husk and bran before cooking or further processing, aiming to decrease antinutrient compounds and improve edibility and sensory quality (Devisetti et al., 2014). However, some important phytochemicals, namely antioxidant phenolic compounds, dietary fibers and micronutrients, were predominantly positioned in husk and bran layers. In our previous research, husk and bran were found to provide far higher content of phenolics and antioxidant activity than the corresponding milled millet products (Zhang et al., 2021). Pradeep and Sreerama (2017) also found that phenolic contents decreased from outer layers to inner layers, in the order of hull > bran > pearled grains for foxtail and little millet grains, and different milled fractions of millets exhibited significant differences ($p < 0.05$) in antioxidant activity. In addition, Li et al. (2022) reported that yellow proso millet bran layer contained higher crude protein, crude fat, and starch content than husk layer. A Heatmap is a form of

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data visualization in which the size or density of a value is represented by the shade or change of color to observe the distribution and trend. It has been reported that phenolic acids, flavonoids and minerals of different varieties of dehusked foxtail millet were visually lower than those of their corresponding husk and bran through cluster heat maps (Li, Zhou, et al., 2024).

Refined process could result in significant differences in content of various nutrients between whole grain and refined grain products, and large amounts of nutrients and health-promoting components should be lost due to excessive milling and further polish. Sunagar and Sreerama (2024) found that protein, fat, carbohydrate, dietary fiber and phytic acid of browntop millet exhibited significant differences ($p < 0.05$) among whole, hulled and pearled millet. And compared with dehusked millet and polished millet, whole millet contains higher content of total phenols, total flavonoids, and dietary fiber in foxtail and little millet (Devisetti et al., 2014). It was also found that the content of antinutrients in pearl millet decreased with the increase of polishing time and there was a strong correlation between crude fiber, protein, phytic acid and tannin by the heatmap of correlation analysis (Joshi & Srinivasa Rao, 2024).

Currently, little research has been reported regarding the changes and differences in nutrients, antinutrients and phytochemicals of foxtail millet with different milling degree and the dynamic process of whole grain being processed into millet. In this study, we want to look for the appropriate milling degree during foxtail millet milling process, for the purpose of maintaining as many nutritional and healthy properties as possible. In this regard, we evaluate the change of major nutrients and the reduction ratio of phytate acid, carotenoid, GABA, free and bound phenolic acids, flavonoids, and antioxidant capacity *in virto* (DPPH, ABTS, FRAP), after being processed with different milling degree.

2. Materials and methods

2.1. Materials and reagents

Ethyl acetate, n-hexane, ethanol, acetone, methanol, sodium nitrite ($NaNO_2$) sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), sodium hydroxide (NaOH), ferric trichloride (FeCl3), aluminum trichloride ($AlCl_3$), sulfuric acid (H_2SO_4), copper sulfate ($CuSO_4$), potassium sulfate (K_2SO_4), petroleum ether, ferric ammonium sulfate, 3, 5-dinitrosalicylic acid, phenol, anhydrous sodium sulfite, and glucose, were all obtained from Tianjin Dean Chemical Reagent Co., Ltd. (Tianjin, China). Thioglycolic acid and 2,2′- bipyridine were obtained from Shandong keyuan Biochemical Co., Ltd. (Shandong, China). Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-Tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), 2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenz-othiazoline-6-sulfonic acid) diammonium salt (ABTS) were sourced from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The standards, lutein, sodium phytate, γ-aminobutyric acid (GABA), *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-coumaric acid, protocatechuic aldehyde, ferulic acid, vanillic acid, caffeic acid, syringic acid, apigenin, kaempferol, and rutin were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Formic acid, HPLC and mass-spectrometry grade methanol were obtained from Thermo Fisher Scientific Reagent Co., Ltd. (Waltham, Massachusetts, USA).

Foxtail millet (Yugu 18 variety) was chosen for this research. To remove the outer layers of the grain (including the husk and bran), the whole foxtail millet grain was processed after harvest by using a separate grain milling machine (6NZF-33, Yutai Quanli Grain Milling Machinery Co., Ltd., Jining, China) through hulling, husk separation, two times milling and polishing processes to obtain the one-time, two-time, three-time milling millet, and polished foxtail millet samples, separately. All the samples with different milling degree were ground into flour, sieved using 40 mesh sieve, and the resulting millet flour was stored at −20 °C for further analysis.

2.2. Determination of crude protein, fat, moisture and ash contents

The protein content was determined by the AOAC standard Kjeldahl method (AOAC 2001.11), the fat content was determined by the Soxhlet method (AOAC 945.16), and the moisture and ash content were determined by the AOAC 2001.12 and AOAC 942.05 method (AOAC, 2005).

2.3. Determination of total starch content

The content of starch in the millet samples was determined by 3, 5-dinitrosalicylic acid colorimetry (Muhammad et al., 2022) with minor modifications. Crude fats and reducing sugars were removed using n-hexane and 85 % ethanol, and then the treated samples were hydrolyzed with 6 mol/L hydrochloric acid solution in a boiling water bath for 30 min. After hydrolysis, the hydrolyzed solution was adjusted to pH 7–7.3 with sodium hydroxide solution, and then reacted with DNS in a boiling water bath for 5 min. After cooling to room temperature, and the absorbance value at 540 nm was read. The glucose standard curve and the conversion factor of 0.9 were used to quantify the total starch content.

2.4. Measurement of color characteristics

The color characteristics of foxtail millet flour were measured with a colorimeter (Color i5, Xrite, USA). L* represents lightness. a* represents redness-greenness, and b* represents yellowness-blueness.

2.5. Determination of total carotenoid content (TCC)

The carotenoid was extracted with 50 % acetone-ethanol solution (*v*/v), for 2 h at a constant temperature of 40 °C, and then centrifuged for 10 min at 10000 r/min to obtain the carotenoid extract (Zhang et al., 2025). The absorbance value of carotenoid extract at 450 nm was determined. A standard curve for carotenoids was established by using lutein as standard, and the results were expressed as milligram of lutein equivalents per 100 g (mg/100 g) of sample.

2.6. Determination of GABA content

GABA was extracted with 80 % methanol, the liquid fraction was dried and redissolved in 3 mL of 50 % methanol solution. The obtained sample solution was gradually diluted 1000 times for UPLC-QQQ-MS analysis.

The UPLC was conducted on a column of Accucore C_{18} (100 mm × 3 mm, Thermo Fisher Scientific, Waltham, USA) at the temperature of 30 °C. The mobile phase consisted of water (A) with 0.1 % formic acid and acetonitrile (B), at the flow rate of 0.2 L/min. The elution program was performed following the previously discussed by Li et al. (2023). The positive ion mode was used for multiple reaction monitoring, and the capillary and cone voltages were 0.35 kV and 30 V, respectively. The ion source and dissolvent gas temperature were set at 150 °C and 500 °C. The collision energy was 4 V. Nitrogen (N_2) and helium (He) were used as dissolvent gas with flow rate of 800 L/h and cone gas with flow rate of 20 L/h, respectively. The GABA quantification of the different milling degree samples was achieved by comparing peak areas against external standards with the ion pairs being monitored at 104.0/87.0 (*m/z*).

2.7. Determination of phytic acid content

The determination of phytic acid was based on the method reported by Sunagar and Sreerama (2024) with minor modifications. In brief, the millet sample (1 g) was extracted using 0.2 mol/L hydrochloric acid (20 mL), and the extract (1.0 mL) was taken and mixed with 2 mL ferric ammonium sulfate solution (0.2 g/L) to keep in a boiling water bath for 30 min. After cooling, it was centrifuged and added with 4 mL 2,2′-bipyridine (10 g/L) to read the absorbance at 519 nm, and sodium

phytate was used as the standard to calculate the phytic acid content of foxtail millets.

2.8. Determination of phenolic content and antioxidant capacity

Extraction of phenolics was conducted according to our published method of Xiang et al. (2019). The millet sample was defatted with hexane (1:10 *w/v*, 15 min) and extracted with 80 % methanol (1:20 w/v, 1 h) by oscillation. The supernatant obtained by centrifugation was the free phenolic fraction. All the above operations were repeated twice. After drying in a fume hood, the remaining pellet was hydrolyzed with 2 mol/L NaOH solution (40 mL) and extracted three times with ethyl acetate (50 mL) to obtain the bound phenolic fraction.

The total phenolic content (TPC) was determined by using the Folin-Ciocalteu colorimetric method reported by Zheng et al. (2022) on a 96-well microplate, with ferulic acid as standard. The sample extract (20 μL) was mixed with Folin-Ciocalteu reagent (40 μL), followed by the addition of 160 μL of 75 g/L Na_2CO_3. After reacting for 1.5 h in the dark, the absorbance was measured at 750 nm. TPC was expressed as milligrams of ferulic acid equivalents per kilogram of dry weight (mg FAE/kg DW). The total flavonoid content (TFC) was determined by $AlCl_3$ colorimetric method reported by Zheng et al. (2022), with rutin as standard. The sample extract (0.4 mL) was mixed with 1.1 mL distilled water, 75 μL of $NaNO_2$ (5 %) and 150 μL of $AlCl_3$ (10 %) for 1 min. After adding 500 μL NaOH (4 %), the solution was diluted to 2.5 mL with distilled water and reacted for 15 min, and the absorbance was measured at 510 nm. TFC was expressed as milligrams of rutin equivalents per kilogram of dry weight (mg RE/kg DW).

The antioxidant capacity of foxtail millet samples with different milling degree was evaluated by three assays, namely DPPH and $ABTS^+$ radical scavenging activity and ferric reducing antioxidant potential (FRAP), according to our previously reported methods of Zheng et al. (2022). The DPPH value is determined by measuring the absorbance at 515 nm of a solution that 10 μL of the extract is mixed with 190 μL of a 60 μM DPPH solution, followed by a 30-min reaction at room temperature. The ABTS value is determined by measuring the absorbance at 750 nm of a solution that 10 μL of the extract is mixed with 190 μL of an $ABTS^+$ radical solution (prepared by diluting an $ABTS^+$ stock solution with ethanol to achieve an absorbance of approximately 0.80 at 750 nm), followed by a 30-min reaction at room temperature. The FRAP value is determined by measuring the absorbance at 593 nm of a solution that 10 μL of the extract is mixed with 300 μL of a ferric-TPTZ reagent (prepared by combining 300 mmol/L acetate buffer, 100 mmol/L TPTZ in 40 mmol/L hydrochloric acid solution, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 *v*/v/v ratio), followed by a 2 h reaction at room temperature. The results were expressed as micromole Trolox equivalents per gram (μmol TE/g) of dry weight sample.

2.9. Identification and quantification of individual polyphenols by UPLC-MS

Ultra-performance liquid chromatography (Waters UPLC H-Class) coupled to triple quadrupole mass spectrometry (Waters Xevo TQ-S/micro) was used for UPLC-MS analyses. During analysis, phenolic extract (10 μL) was injected and eluted through the C_{18} column (100 mm × 3 mm, Thermo Fisher Scientific, Waltham, USA). The column temperature was set at 35 °C. The mobile phase was composed of solvent A (ultrapure water containing 0.1 % formic acid) and solvent B (MS grade methanol containing 0.1 % formic acid). The gradient elution procedure, flow rate and settings on the mass spectrometer were consistent with our previously described method of Xiang et al. (2023).

Phenolic compositions were identified by comparing UV spectrum characteristic and MS/MS information from UPLC-MS/MS. The contents of individual phenolic compounds were calculated by using external standard method. The detection of *p*-hydroxybenzoic acid derivatives, including syringic acid, protocatechuic aldehyde, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid and vanillic acid, were conducted at 280 nm. The detection of hydroxycinnamic acid derivatives, such as ferulic acid, *p*-coumaric acid and sinapic acid, were conducted at 320 nm. The detection of flavonoids, for example, apigenin and kaempferol, were conducted at 350 nm. The results were expressed as mg per kg (mg/kg).

2.10. Statistical analysis

All the analyses were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). The differences of mean values among milled millets were evaluated by using ANOVA followed by Tukey's HSD test at a significance level of $p < 0.05$. All the data were processed using SPSS software for Windows version 26.0. Origin 2021 was used to carry out Pearson's correlation analysis.

3. Results and discussion

3.1. Effect of milling degree on proximate composition, total carotenoid content and color characteristics of foxtail millet

The effects of milling degree on the total starch, crude protein, crude fat and ash contents of foxtail millet samples are presented in Table 1. As an important low glycemic index (GI) food material, foxtail millet is generally consumed as porridge or cooked millet, and plays an important part in improving glucostasis by generating low level of postprandial blood glucose (Anitha et al., 2021). The total starch content significantly ($p < 0.05$) increased with the increase of milling degree, and the polished foxtail millet sample reached the highest level of 71.81 % (*w*/w), which was 3.58 times as that of whole foxtail millet grain. The higher percentage of starch produces more glucose, which could lead to an increase in glycemic index (Demangeat et al., 2023). In addition, milling could lead to the loss of polyphenols, which could inhibit α-amylase activity, resulting in the increase of starch digestibility and glycemic index (Alonso et al., 2000). It suggests that high milling degree may reduce the potential of foxtail millet as a low GI food material.

The contents of moisture, crude fat and crude protein firstly

Table 1
The nutrients and color characteristics of the foxtail millets with different milling degree.

Levels	Whole millet	One-time milling millet	Two-time milling millet	Three-time milling millet	Polished millet
Moisture (%)	9.11 ± 0.01^{d}	9.79 ± 0.02^{a}	9.83 ± 0.03^{a}	9.72 ± 0.01^{b}	9.35 ± 0.04^{c}
Starch (%)	20.05 ± 0.51^{e}	35.03 ± 0.76^{d}	51.98 ± 0.65^{c}	62.37 ± 0.47^{b}	71.81 ± 0.46^{a}
Fat (%)	3.50 ± 0.10^{c}	4.52 ± 0.05^{a}	4.35 ± 0.09^{a}	3.90 ± 0.17^{b}	3.55 ± 0.09^{c}
Protein (%)	11.38 ± 0.02^{d}	12.39 ± 0.08^{b}	12.72 ± 0.14^{a}	12.26 ± 0.09^{b}	12.06 ± 0.04^{c}
Ash (%)	2.54 ± 0.02^{a}	1.69 ± 0.21^{b}	1.37 ± 0.05^{c}	1.20 ± 0.03^{d}	1.18 ± 0.02^{d}
Carotenoid (mg/100 g)	1.48 ± 0.01^{e}	1.82 ± 0.04^{d}	2.12 ± 0.03^{a}	2.02 ± 0.01^{b}	1.90 ± 0.04^{c}
Phytic acid (mg/g)	13.96 ± 0.16^{b}	15.40 ± 0.03^{a}	15.74 ± 0.12^{a}	13.20 ± 0.08^{c}	6.04 ± 0.13^{d}
L*	79.27 ± 0.25^{e}	81.56 ± 0.35^{d}	82.54 ± 0.47^{c}	83.81 ± 0.13^{b}	85.72 ± 0.08^{a}
a*	3.32 ± 0.02^{a}	3.28 ± 0.01^{a}	3.30 ± 0.03^{a}	3.27 ± 0.06^{a}	2.72 ± 0.03^{b}
b*	21.63 ± 0.13^{d}	25.29 ± 0.15^{c}	28.32 ± 0.13^{a}	27.94 ± 0.07^{ab}	27.85 ± 0.10^{b}
ΔE	–	4.34 ± 0.23^{c}	7.48 ± 0.12^{b}	7.78 ± 0.08^{b}	8.89 ± 0.06^{a}

Results are expressed as mean ± SD. Different letters with each column indicate significant differences ($p < 0.05$).

increased and then decreased with the increase of milling degree. From whole millet to two-time milling millet, the moisture content increased from 9.11 % to 9.83 %, and then decreased to 9.35 % in polished millet. The change of moisture content may be closely related to the difference of moisture content in different parts of millet (Li et al., 2022; Sunagar & Sreerama, 2024). In addition, the mechanical and frictional heat generated during milling may also accelerate the evaporation of water, thereby further reducing the moisture content (Kalpanadevi et al., 2018). The crude fat content of the polished millet decreased to 3.55 %, which is significantly 21.46 % lower ($p < 0.05$) than the one-time milling millet sample with the highest level of 4.52 %. More than 80 % of the fatty acids in millet fat are unsaturated fatty acids, which have hypoglycemic and hypolipidemic effects (Sharma & Niranjan, 2017). In addition, the fat in millet is released during cooking, which could increase the sensory quality. The crude protein content of the polished millet decreased to 12.06 %, which is significantly 5.19 % lower ($p < 0.05$) than the two-time milling millet sample with the highest content of 12.72 %. The foxtail millet protein is easily digested by human body and also displays positive effects on type 2 diabetes and cardiovascular disease (Sharma & Niranjan, 2017). In addition, the combination of protein or fat with other factors, such as ready digestible carbohydrates, amylases, will reduce the digestion rate in small intestine, resulting in prolonged or incomplete digestion of starch, which has positive effects on low GI property (Anitha et al., 2021). Therefore, appropriate milling degree can not only increase the fat and protein content, which are beneficial to human health, but also improve the sensory quality of millet. These performances were mainly attributed to that large amounts of fat and protein of foxtail millet were mainly included in the bran layer, while starch mainly existed in the endosperm of millet grains (Li et al., 2022). Minerals were mainly found in the husk of millet seeds, and total mineral content should decrease with de-husk and de-bran process (Li, Cai, et al., 2024). Therefore, the ash content of foxtail millet reduced to 1.18 %, decreasing by 53.54 % when compared with the whole millet. The impact of milling on the proximate composition of millet has also been observed in other types of millet. For example, Niranjan and Dwivedi (2022) reported that the moisture, protein, and fat content of the hulled browntop millet flour (BTMB) were higher than in whole browntop millet flour (BTMU) and polished browntop millet flour (BTMP), while the highest ash content was found in BTMU, followed by BTMB, and then BTMP. Similarly, in pearl millet, polishing resulted in an increase in carbohydrate content and a decrease in protein, ash, and fat content, with longer polishing times leading to greater changes in composition (Joshi & Srinivasa Rao, 2024). In barnyard millet, milling time also caused a reduction in proximate composition, with protein, fat, ash, and fiber showing a linear negative correlation with the degree of polishing (Lohani et al., 2011).

For the color characteristics, the increasing of the milling degree caused an increase in the lightness of the millet, which may be due to the removal of outer nutrients during the milling process, mainly phenolic compound, the fat-soluble and the mineral nutrients (Joshi & Srinivasa Rao, 2024; Li et al., 2022). As the degree of milling increased, the a* value showed an overall decreasing trend, while b* value presented first increasing and then decreasing trend, and from whole millet to two-time milling millet, the b* value increased from 21.63 to 28.32 and subsequently decreased to 27.85 in polished millet. These results were in accordance with those previously reported by Li et al. (2022), who indicated that the a* value of yellow proso millet husk was higher than that of bran, while b* value of yellow proso millet bran was higher than that of husk. Similarly, in the study by Niranjan and Dwivedi (2022) on hulled (BTMB), polished (BTMP), and whole (BTMU) browntop millet flour, BTMU exhibited the smallest L* and b* values, followed by BTMB and BTMP. Additionally, BTMU had the largest a* value, followed by BTMB and BTMP. Milling leads to an increase in the total color difference (ΔE), with a maximum change of 4.34 observed after one-time milling process, which represents the largest variation between adjacent samples. This indicates that the removal of the husk is the primary factor contributing to the change in ΔE.

The total carotenoid content (TCC) of foxtail millet samples exhibited a significant difference ($p < 0.05$) and showed an overall trend of first increasing and then decreasing with the increase of milling degree, which may be because a large proportion of carotenoids were existing in the bran layer. From whole millet to two-time milling millet, TCC increased from 1.48 to 2.12 g/100 g with the increasing percentage of 43.24 %, and subsequently decreased to 1.90 g/100 g in polished millet with the loss percentage of 10.38 %. Krishnan and Meera (2021) found that the removal of bran would lead to the decrease of β-carotene content in pearl millet grain, and the β-carotene content in the middle layer of the pericarp was higher, which gradually decreased to the inner layer of the grain. The abundant carotenoids in foxtail millet generally contribute to the color of grain seeds (Li et al., 2022). The highest TCC and the maximum b* value were meanwhile displayed in the two-time milling millet sample with the level of 2.12 mg/100 g and the value of 28.32, respectively. Our results demonstrated that the crude protein, crude fat, ash and total carotenoids of foxtail millet would be lost with varying degrees during milling process.

3.2. Effect of milling degree on phytic acid

Phytic acid (PA) was generally believed as an anti-nutrient widely presented in cereal grains, because it could bind to minerals such as iron, copper, zinc, and calcium, hindering their absorption. However, Food and Drug Administration (FDA) now classifies PA as a recognized safe product. PA is also regarded as a nutritional supplement with antioxidant, anti-inflammatory, and blood-sugar regulating properties, and is beneficial to prevention of a variety of chronic diseases by scavenging free radicals, suppressing inflammation, and regulating insulin (Li, Zhou, et al., 2024).

The phytic acid contents of foxtail millets with different milling degree are presented in Table 1, which shows a first increase and then decreased trend in phytic acid level with the increase of milling degree. From the whole millet to polished millet, the phytic acid decreased from 13.96 mg/g to 6.04 mg/g, with the loss percentage of 56.73 %. No significant difference ($p < 0.05$) between the one-time milling millet and the two-time milling millet was observed, and the highest level of 15.74 mg/g was displayed in the two-time milling millet. It is probably because that phytic acid is mainly present in the bran layer of millet grain (Sunagar & Sreerama, 2024). This result is consistent with the result of proso millet reported by Devisetti et al. (2014), who found that both polished millet and whole millet samples displayed lower phytic acid levels than brown millet with the highest content of 8.3 mg/g. Singh and Rao (2025) also reported that the phytate content of browntop millet decreased from 1.04 to 0.81 mg/g after hulling, with a loss of 22.11 %. Additionally, Joshi and Srinivasa Rao (2024) found that polishing pearl millet led to a reduction in phytate content, with longer polishing times resulting in greater phytate loss.

3.3. Effect of milling degree on GABA content of foxtail millet

γ-aminobutyric acid (GABA) is a naturally occurring potentially bioactive compound and widely distributed in a variety of natural foods, primarily including grains, beans, fruits, and vegetables. GABA-enriched foods have wonderful health benefits for humans, including neuroprotection, anti-insomnia, anti-diabetes, anti-depression, anti-hypertension and anti-inflammatory (Hou et al., 2023). The GABA content of foxtail millet after treatment with different milling degree is presented in Fig. 1, which showed that GABA gradually reduced with the increase of milling degree. From the whole millet to the polished millet sample, the level of GABA decreased from 38.10 mg/kg to 17.43 mg/kg, with the loss percentage of 54.25 %. The gradual loss of GABA was attributed to variations of GABA in different partitions of millet grain seeds. With the removal of husk, bran and some proportions of endosperm of the millet structure, GABA was removed and lost, and the content decreased

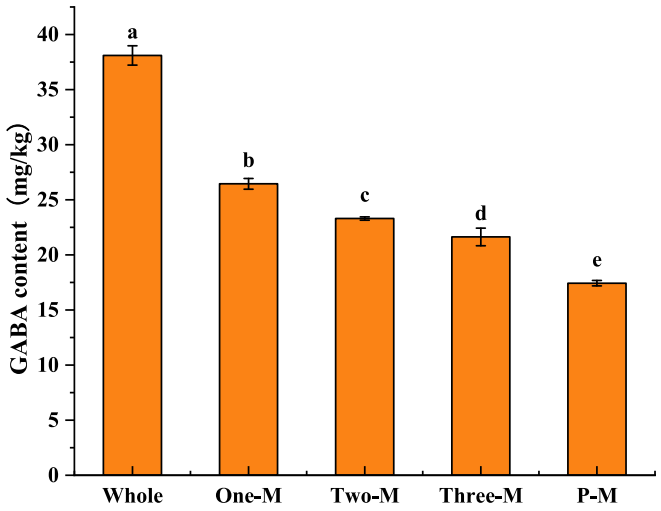

Fig. 1. The GABA content of foxtail millets with different milling degree. Whole, One-M, Two-M, Three-M and P-M are the obtained millet samples from different milling degree; Whole, whole foxtail millet; One-M, one-time milling millet; Two-M, two-time milling millet; Three-M, three-time milling millet; P-M, polished millet. Different letters indicate significant difference ($p < 0.05$).

correspondingly. This was consistent with the reported results of other cereal grains. For example, milling process resulted in loss of GABA in rice grain, unmilled whole brown rice generally exhibited significantly ($p < 0.05$) higher GABA content compared to corresponding milled products (Shin et al., 2022).

3.4. Effect of milling degree on polyphenol content of foxtail millet

Fig. 2 presents the levels and loss percentages of the free and bound phenolics of foxtail millets with different milling degree. The free, bound, and total phenolic and flavonoid contents exhibited significant differences ($p < 0.05$) among the foxtail millet samples, and all of them shared a similar decreasing trend. As milling degree increased, compared with whole millet with free TPC of 976.64 mg/kg DW and bound TPC of 3081.03 mg/kg DW, the loss percentages of free TPC were 42.78 %, 57.48 %, 60.00 % and 69.79 %, and the loss percentage of bound TPC were 61.98 %, 76.33 %, 76.86 % and 83.96 %, respectively. The total content of phenolics (free and bound forms) of whole millet was 4057.67 mg FAE/kg, which was significantly 5.1 times higher ($p < 0.05$) than those of the polished millet sample.

The whole grain of foxtail millet contained abundant natural phenolic compounds contributing to its health benefits, while significantly ($p < 0.05$) reduced with the increase of milling degree, although highly fine milling improved the sensory quality of millet. The

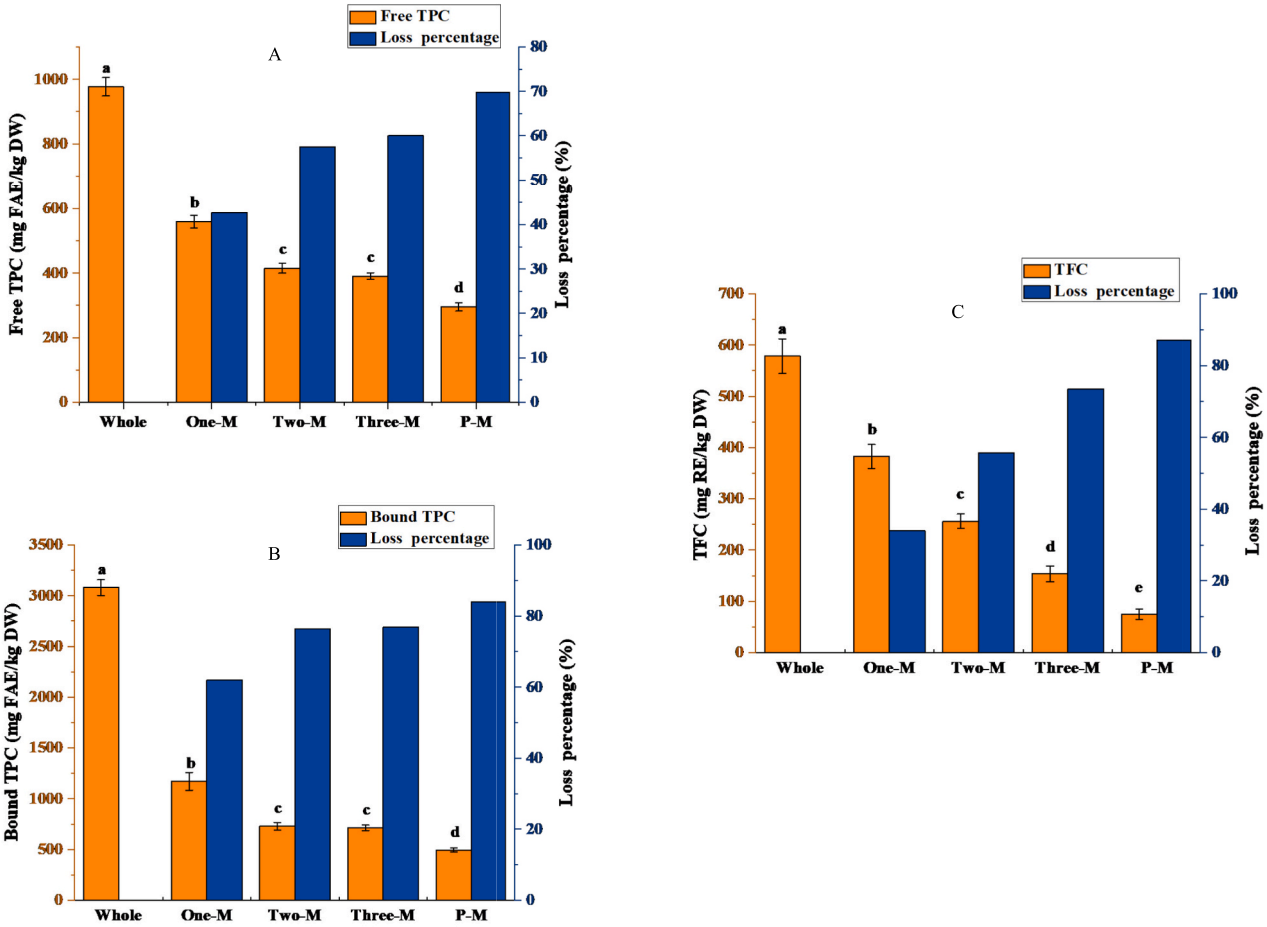

Fig. 2. The phenolic contents and the loss percentage of the foxtail millets with different milling degree. A: free total phenolic content (free TPC), B: bound total phenolic content (bound TPC), C: total flavonoid content (TFC), Whole, One-M, Two-M, Three-M and P-M are the obtained foxtail millet samples from different milling degree; Whole, whole millet; One-M, one-time milling millet; Two-M, two-time milling millet; Three-M, three-time milling millet; P-M, polished millet. Different letters at the top of the columns indicate significant difference ($p < 0.05$). DW, dry basis weight.

performance was also observed in two foxtail millets and one proso millet from India reported by Devisetti et al. (2014), who found that the TPC of whole grain of *PS-4* foxtail millet, *SIA-3126* foxtail millet and proso millet were 2.1 fold, 2.32 fold and 3 fold as their corresponding brown millet samples, and 3.4 fold, 3.15 fold and 5 fold as their corresponding polished millet samples, respectively. For the different kinds of millet, including kodo millet, figer millet, foxtail millet, proso millet, little millet, pearl millet, Chandrasekara et al. (2012) reported that the total phenolic content of whole millets was significantly higher ($p < 0.05$) than that of dehulled millets. The highest phenolic loss percentage was observed in the one-time milling millet, which could be inferred that the phenolic compounds in millet seeds mainly existed in the husk layer, which was in agreement with the previous finding (Li et al., 2022). In addition, phenolic components, as a class of thermosensitive phytochemicals, could probably be influenced and lost by thermal degradation, mechanical friction or interaction with other chemical constituents (Walter et al., 2013).

The TFC of foxtail millet shared similar decreasing trend with TPC, as milling degree increased. The whole millet presented the highest TFC level of 578.33 mg RE/kg, which was significantly ($p < 0.05$) decreased by 33.86 %–87.09 % with the increasing of milling degree. Flavonoids, as one of the most important phenolic components, were also mainly distributed in the husk and bran of foxtail millet grains (Zhang et al., 2021). In addition, relatively high friction temperature during mechanical processing can lead to the decomposition of flavonoid compounds and the change of molecular structure, which results in reduction of flavonoids (Jiang et al., 2022). Similar performance was observed on little millet that the highest TFC was displayed in whole millet, followed by dehulled and pearled millet (Pradeep & Sreerama, 2017). During rice milling, similar results were also reported that *Dao Huaxiang* rice and *Jiangxi Indica* rice from China presented 43.3 %–72.9 % and 58.7 %–87.7 % loss of flavonoids (Ma et al., 2020).

3.5. Effects of milling degree on phenolic profiles and individual phenolic contents

3.5.1. Individual free phenolic compounds

The presentative free phenolic profile of foxtail millet is displayed in Fig. 3(A). Nineteen phenolic compounds in free form were identified by analyzing their UV spectral characteristics and the obtained MS/MS fragment information, and then comparing with the authentic standards or our previously published data (Xiang et al., 2019; Zhang et al., 2021; Zheng et al., 2022). The free phenolic compounds were identified as *p*-hydroxybenzoic acid (peak 1), protocatechuic aldehyde (peak 2), *p*-hydroxybenzaldehyde (peak 3), vanillic acid (peak 4), syringic acid (peak 5), *trans*-*p*-coumaric acid (peak 6), 1-O-*p*-coumaroylglycerol (peak 7), apigenin-C-dihexoside (peak 8), *trans*-ferulic acid (peak 9), N′-*p*-coumaroyl-N″-caffeoylspermidine (peak 10), apigenin-C-pentosyl-C-hexoside (peak 11), N′,N″-di-*p*-coumaroylspermidin (peak 12), apigenin-C-pentosyl-C-hexoside isomer (peak 13), kaempferol-C,O-

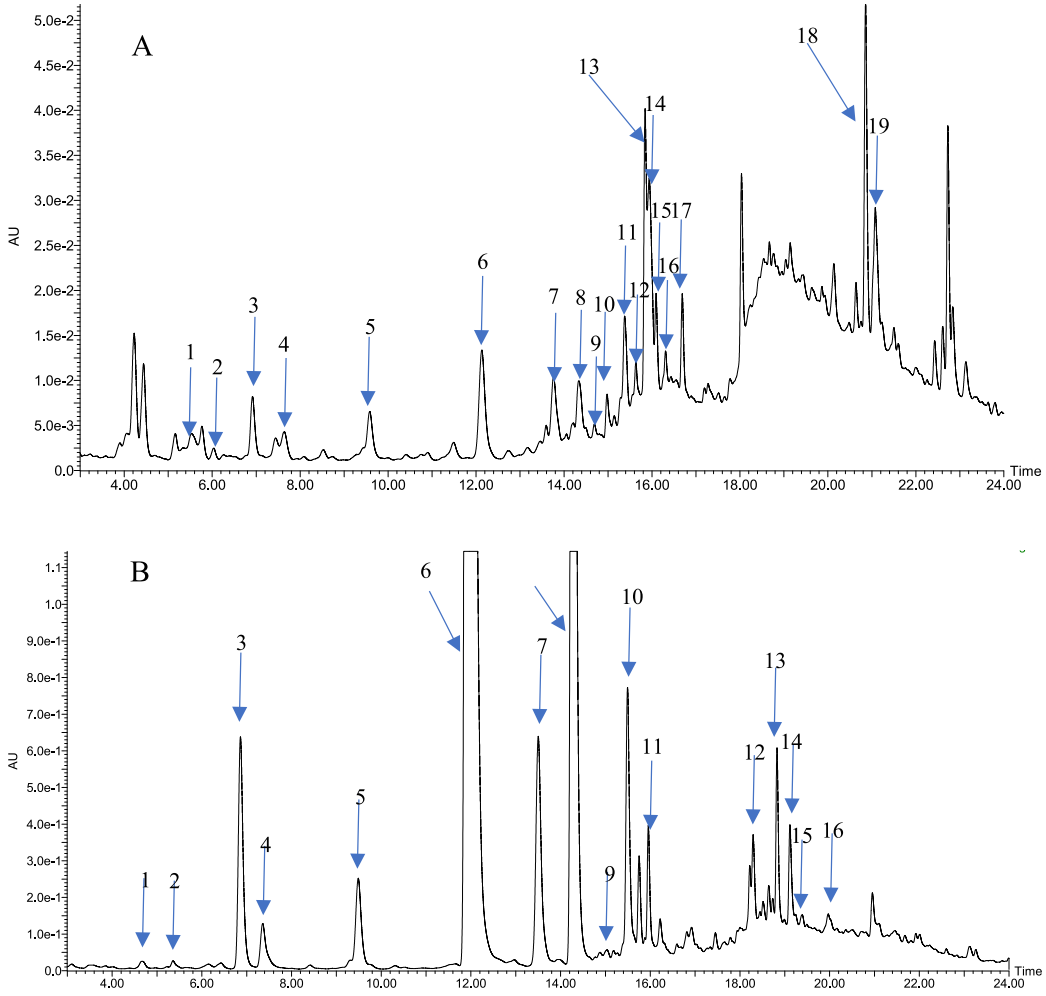

Fig. 3. UPLC profiles of soluble free and insoluble bound phenolic compounds of whole foxtail millet. Detection was set at wavelength of 280 nm for free phenolic fractions (A) and bound phenolic fractions (B). Phenolic compounds were confirmed by comparing with standards or putatively identified by UV spectra, MS and MS/MS data.

dihexoside (peak 14), N′-*p*-coumaroyl-N″-feruloylspermidine (peak 15), di-feruloylspermidine (peak 16), apigenin-C-pentosyl-C-hexoside isomer (peak 17), 1-O-*p*-coumaroyl-3-O-feruloylglycerol (peak 18) and 3,7-dimethyiquercetin (peak 19), respectively.

The effects of milling degree on the free phenolic levels of foxtail millets are shown in Table 2. With the increased milling degree of foxtail millet, except for N′-*p*-coumaroyl-N″-caffeoylspermidine, the content of the individual free phenolic compounds decreased significantly ($p < 0.05$), which resulted in the change of phenolic profile. Among these phenolic compounds, *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde reduced with the increase of milling degree and were undetectable after being polished. 1-O-*p*-coumaroylglycerol, only present in free form, decreased from 5.00 to 0.25 mg/kg DW during the milling process. The contents of protocatechuic aldehyde, vanillic acid, trans-*p*-coumaric acid, syringic acid and *trans*-ferulic acid significantly ($p < 0.05$) decreased from 10.33 to 0.61, 12.25 to 6.74, 7.66 to 1.32, 3.99 to 0.45 and 6.30 to 2.33 mg/kg DW, and the loss rates reached 94.09 %, 44.98 %, 82.77 %, 88.72 % and 63.01 %, respectively. The reduction of these phenolic compounds may be due to the fact that phenolic acids are mainly present in the husk and bran of foxtail millet (Li et al., 2022; Zhang et al., 2021).

Flavonoids, the polyphenols characterized by the C6-C3-C6 structure, are widely distributed in plant foods, and exert their effects through different mechanisms, such as scavenging reactive oxygen species, modulating enzyme activities and interacting with cellular signaling pathways (Jakobek & Blesso, 2023; Karakaya, 2004). As milling degree increased, all the individual flavonoid compounds significantly ($p < 0.05$) decreased. Apigenin-C-dihexoside and apigenin-C-pentosyl-C-hexoside isomers were becoming undetectable in the polished millet, and the contents of kaempferol-C, O-dihexoside and 3,7-dimethyiquercetin were reduced from 17.16 to 1.19 mg/kg DW and 19.67 to 3.95 mg/kg DW, with their loss rates of 93.06 % and 79.92 %, respectively, from whole millet grain to polished millet. This was similar to the results of Pradeep and Sreerama (2017) that the apigenin and kaempferol showed significant decreasing trend after the whole foxtail millet grains being milled into pearled millet. The reduction pattern of these flavonoids from the different milling degree suggested that kaempferol-C, O-dihexoside mainly existed in the millet bran, and apigenin-C-dihexoside, apigenin-C-apigenin-C-pentosyl-C-hexoside and 3,7-dimethyiquercetin mainly existed in the husk of foxtail millet seeds. Previous reports about different milling fractions of foxtail millet also showed that the content of apigenin in husk was significantly higher ($p < 0.05$) than that in bran (Pradeep & Sreerama, 2017), and the content of kaempferol in bran was significantly higher ($p < 0.05$) than that in husk (Zhang et al., 2021).

3.5.2. Individual bound phenolic compounds

The presentative bound phenolic profile of foxtail millet is displayed in Fig. 3(B). The sixteen bound phenolic compounds were identified by the authentic standards or our previously published data (Li et al., 2023; Xiang et al., 2019; Zhang et al., 2021). The bound phenolic compounds were assigned as protocatechuic aldehyde (peak 1), *p*-hydroxybenzoic acid (peak 2), *p*-hydroxybenzaldehyde (peak 3), vanillic acid (peak 4), syringic acid (peak 5), *trans*-*p*-coumaric acid (peak 6), *cis*-*p*-coumaric

Table 2
The individual free and bound phenolic contents of the foxtail millets with different milling degree.

	No.	Phenolic compositions	Whole millet (mg/kg DW)	One-time milling millet (mg/kg DW)	Two-time milling millet (mg/kg DW)	Three-time milling millet (mg/kg DW)	Polished millet (mg/kg DW)
Free	1	*p*-Hydroxybenzoic acid	3.85 ± 0.23^{a}	3.39 ± 0.13^{b}	2.94 ± 0.51^{c}	2.64 ± 0.12^{d}	n.d.
	2	Protocatechuic aldehyde	10.33 ± 0.45^{a}	8.58 ± 0.98^{b}	7.59 ± 0.07^{b}	5.05 ± 0.45^{c}	0.61 ± 0.05^{d}
	3	*p*-Hydroxybenzaldehyde	13.42 ± 1.23^{a}	7.76 ± 0.06^{b}	5.17 ± 0.21^{c}	4.53 ± 0.79^{c}	n.d.
	4	Vanillic acid	12.25 ± 0.13^{c}	16.50 ± 0.18^{a}	14.55 ± 0.29^{b}	9.11 ± 0.57^{d}	6.74 ± 0.45^{e}
	5	Syringic acid	3.99 ± 0.05^{a}	1.86 ± 0.01^{b}	1.62 ± 0.02^{b}	0.81 ± 0.09^{c}	0.45 ± 0.22^{d}
	6	*trans-p*-Coumaric acid	7.66 ± 0.36^{a}	3.82 ± 0.45^{b}	2.86 ± 0.05^{c}	1.81 ± 0.05^{d}	1.32 ± 0.25^{de}
	7	1-*O-p*-Coumaroylglycerol	5.00 ± 0.98^{a}	1.73 ± 0.23^{b}	0.92 ± 0.01	0.41 ± 0.01^{d}	0.25 ± 0.09^{de}
	8	Apigenin-C-dihexoside	2.86 ± 0.89^{a}	0.73 ± 0.05^{b}	0.80 ± 0.01^{b}	0.45 ± 0.03^{c}	n.d.
	9	*trans*-Ferulic acid	6.30 ± 0.06^{a}	5.27 ± 0.86^{b}	5.20 ± 0.22^{b}	3.60 ± 0.15^{c}	2.33 ± 0.20^{d}
	10	N′-*p*-coumaroyl-N″-caffeoylspermidine	1.35 ± 0.02^{b}	1.38 ± 0.03^{b}	1.45 ± 0.02^{a}	1.46 ± 0.01^{a}	1.43 ± 0.01^{a}
	11	Apigenin-C-pentosyl-C-hexoside	4.56 ± 0.34^{a}	1.81 ± 0.67^{b}	1.37 ± 0.09^{c}	0.82 ± 0.06^{d}	n.d.
	12	N′, N ″-di-*p*-coumaroylspermidine	1.47 ± 0.02^{a}	1.09 ± 0.01^{b}	1.03 ± 0.02^{c}	1.00 ± 0.01^{cd}	0.96 ± 0.03^{d}
	13	Apigenin-C-pentosyl-C-hexoside isomer	8.28 ± 0.73^{a}	2.17 ± 0.04^{b}	1.92 ± 0.08^{b}	1.17 ± 0.03^{c}	n.d.
	14	Kaempferol-C, O-dihexoside	17.16 ± 1.02^{a}	17.56 ± 1.34^{a}	12.32 ± 1.34^{b}	8.46 ± 1.54^{c}	1.19 ± 0.01^{d}
	15	N′-*p*-coumaroyl-N″-feruloylspermidine	0.63 ± 0.08^{a}	0.61 ± 0.04^{a}	0.56 ± 0.01^{ab}	0.49 ± 0.01^{b}	0.41 ± 0.02^{c}
	17	Apigenin-C-pentosyl-C-hexoside isomer	4.43 ± 0.21^{a}	1.17 ± 0.03^{b}	0.38 ± 0.01^{c}	0.25 ± 0.02^{cd}	n.d.
	18	3,7-Dimethyiquercetin	19.67 ± 2.32^{a}	6.41 ± 0.34^{b}	6.34 ± 0.22^{b}	4.26 ± 0.08^{c}	3.95 ± 0.12^{c}
Bound	1	Protocatechuic aldehyde	1.07 ± 0.04^{a}	0.33 ± 0.08^{b}	0.20 ± 0.03^{c}	0.12 ± 0.01^{d}	0.06 ± 0.01^{d}
	2	*p*-Hydroxybenzoic acid	1.84 ± 0.10^{a}	1.68 ± 0.13^{b}	0.94 ± 0.02^{c}	0.89 ± 0.05^{c}	0.35 ± 0.08^{d}
	3	*p*-Hydroxybenzaldehyde	132.80 ± 5.21^{a}	98.80 ± 3.54^{b}	71.10 ± 0.56^{c}	58.60 ± 0.42^{d}	24.75 ± 2.62^{e}
	4	Vanillic acid	22.27 ± 3.12^{a}	6.14 ± 0.97^{b}	1.74 ± 0.07^{c}	n.d.	n.d.
	5	Syringic acid	50.72 ± 1.45^{a}	21.30 ± 2.32^{b}	14.10 ± 0.13^{c}	7.16 ± 0.65^{d}	3.82 ± 0.38^{e}
	6	*trans-p*-Coumaric acid	1050.46 ± 8.25^{a}	637.78 ± 15.48^{b}	349.45 ± 5.23^{c}	226.78 ± 2.56^{d}	138.14 ± 5.40^{e}
	7	*cis-p*-Coumaric acid	52.79 ± 2.52^{a}	25.36 ± 0.67^{b}	14.53 ± 0.35^{c}	9.25 ± 0.78^{d}	4.82 ± 0.27^{e}
	8	*trans*-Ferulic acid	377.86 ± 2.96^{a}	228.45 ± 9.34^{b}	168.60 ± 12.02^{c}	164.30 ± 8.34^{c}	120.23 ± 2.59^{d}
	9	Sinapic acid	5.89 ± 0.25^{a}	4.71 ± 0.31^{b}	3.54 ± 0.18^{c}	3.31 ± 0.12^{cd}	2.99 ± 0.05^{d}
	10	*cis*-Ferulic acid	46.20 ± 1.23^{a}	42.11 ± 3.65^{ab}	37.78 ± 1.76^{bc}	36.14 ± 3.10^{cd}	32.11 ± 2.87^{d}
	11	Dihydroferulic acid dimer	22.74 ± 1.88^{a}	10.91 ± 0.34^{b}	8.01 ± 0.13^{c}	7.41 ± 0.22^{d}	7.15 ± 0.19^{d}
	12	8,8′-aryltetralin-DFA	15.15 ± 1.35^{a}	8.47 ± 1.02^{b}	4.95 ± 0.21	3.87 ± 0.38^{d}	2.86 ± 0.29^{e}
	13	*trans-trans*-8-O-4'-DFA	27.53 ± 0.56^{a}	11.21 ± 1.02^{b}	7.4 ± 0.25^{c}	6.6 ± 0.73^{c}	4.26 ± 0.31^{d}
	14	8–5′-DFA	17.14 ± 0.67^{a}	11.02 ± 0.09^{b}	8.97 ± 0.09^{c}	5.23 ± 0.56^{d}	3.43 ± 0.67^{e}
	15	*trans-cis*-8-O-4'-DFA	10.15 ± 0.35^{a}	8.47 ± 1.02^{a}	4.95 ± 0.21^{b}	3.87 ± 0.38^{c}	2.86 ± 0.29^{d}

Values with no letters in common are significantly different ($p < 0.05$). n.d., not detected. DFA: ferulic acid dimer. DW, dry weight of sample. Results are expressed as mean ± SD.

acid (peak 7), *trans*-ferulic acid (peak 8), sinapic acid (peak 9), *cis*-ferulic acid (peak 10), dihydroferulic acid dimer (peak 11), 8,8′-aryltetralin-DFA (peak 12), *trans-trans*-8-O-4'-DFA (peak 13), 8–5′-DFA (peak 14), *trans-cis*-8-O-4'-DFA (peak 15) and ferulic acid dehydrotrimers (peak 16), respectively.

The bound phenolic contents of foxtail millet samples with different milling degree are shown in Table 2. Phenolic acids are predominantly present in bound form in most cereal grains, which are integral components of the cell wall and linked through ester bonds to cellulose, lignin, and proteins (Karakaya, 2004). These bound phenolic derivatives could not be digested by human gastrointestinal enzymes, resulting in survival from gastrointestinal digestion, and released in the colon through fermentation of colonic microflora, providing unique health benefits for humans. (Jakobek & Blesso, 2023). Ferulic and *p*-coumaric acids were the most abundant phenolic acids in bound, which were significantly ($p < 0.05$) reduced with the increase of milling degree, *trans*-ferulic acid and *cis*-ferulic acid decreased from 377.86 to 120.23 mg/kg DW and 46.20 to 32.11 mg/kg DW, with their loss rates of 68.18 % and 30.50 %, respectively. The *trans-p*-coumaric acid and *cis-p*-coumaric acid decreased from 1050.46 to 138.14 mg/kg DW and 52.79 to 4.82 mg/kg DW, with their loss rates of 86.85 % and 90.87 %, respectively. With the increase of milling degree, *p*-hydroxybenzaldehyde and syringic acid decreased from 132.80 to 24.75 mg/kg DW and 50.72 to 3.82 mg/kg DW, respectively. Particularly, vanillic acid was reduced from 22.27 mg/kg DW to undetectable in the three-time milling millet sample. It was indicated that the loss degree of individual bound phenolic acids of foxtail millet was varying for different milling degree, which may be due to a nonuniform distribution of these individual phenolic components in foxtail millet grain. This result was also reflected in different milling fractions of foxtail millet studied by Zhang et al. (2021), where individual bound phenolic compounds exhibited significant differences ($p < 0.05$) among the milling fractions, and the husks had the highest level of individual bound phenolic compounds, followed by the brans and millets. In addition, it has been found in other grains, Liu et al. (2015) reported that the content of coumaric acid, vanillic acid, chlorogenic acid, ferulic acid, isoferulic acid, protocatechuic acid and caffeic acid decreased by 89.8 %, 94.0 %, 80.0 %, 70.8 %, 59.0 %, 56.7 % and 61.3 %, respectively, as the milling degree of *Japonica* rice increased from 0 to 9.49 %.

3.6. Effect of milling degree on antioxidant capacity of foxtail millet

The DPPH, ABTS, and FRAP values of free and bound phenolics from the foxtail millet samples with different milling degree are shown in Fig. 4. DPPH, ABTS^{+} radical scavenging activities, and FRAP of free and bound phenolics exhibited significant differences ($p < 0.05$) among the millet samples and decreased significantly ($p < 0.05$) as milling degree increased. The DPPH values of free, bound, and the sum decreased from

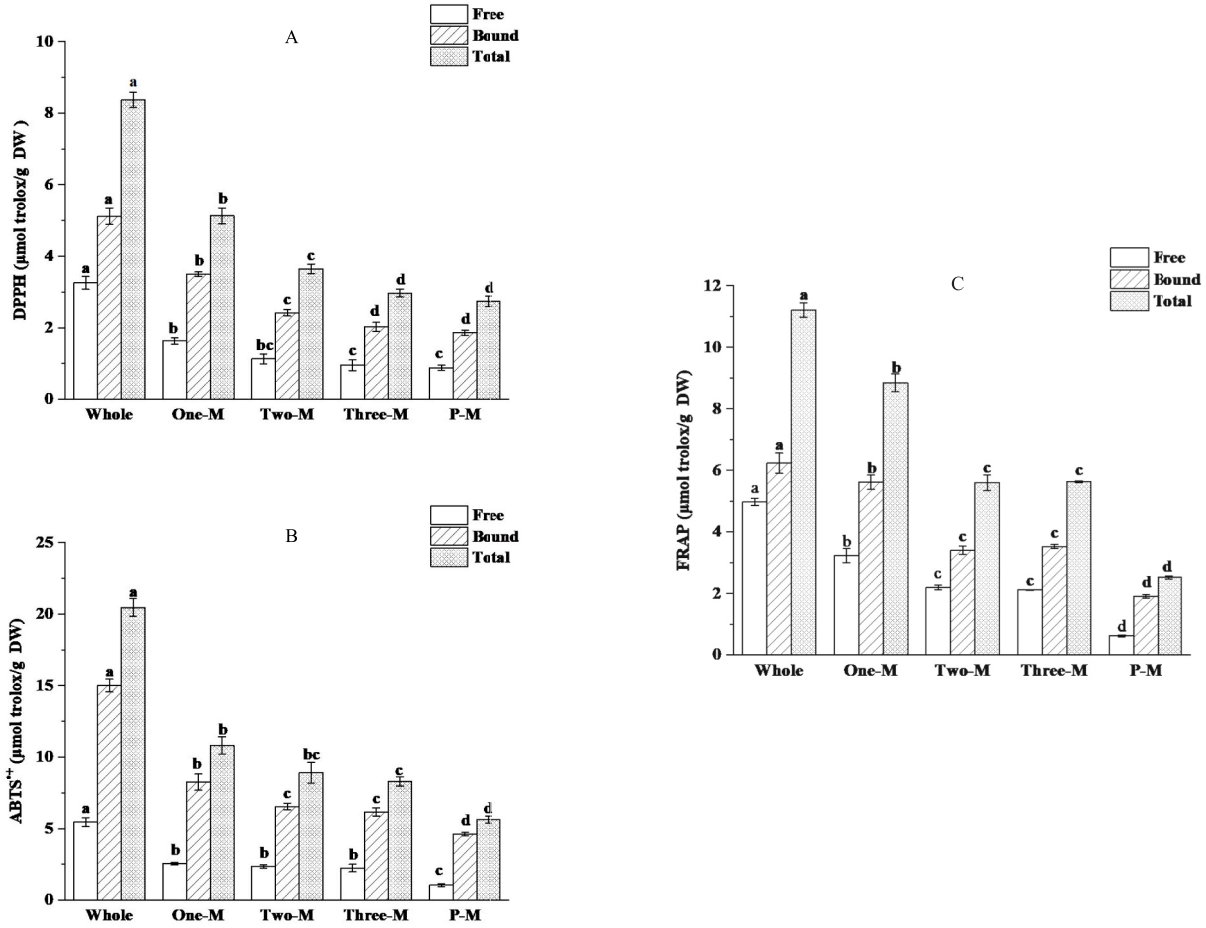

Fig. 4. The scavenging DPPH (A) and ABTS^{+} (B) radical scavenging activities, and FRAP (C) of free and bound phenolics from foxtail millets with different milling degree. Whole, One-M, Two-M, Three-M and P-M are the obtained millet from different milling processes; Whole, whole millet; One-M, one-time milling millet; Two-M, two-time milling millet; Three-M, three-time milling millet; P-M, polished millet. Different letters at the top of the columns indicate significant difference ($p < 0.05$). DW, dry basis weight.

3.25 to 0.88 μmol TE/g DW, 5.12 to 1.86 μmol TE/g DW and 8.37 to 2.74 μmol TE/g DW, respectively, which were shown in Fig. 4(A). And the DPPH values decreased by 72.92 %, 63.67 % and 67.26 %, respectively. From Fig. 4(B) and 4(C), ABTS and FRAP values shared similar significant ($p < 0.05$) decrease performance as milling degree increased, which were consistent with the reducing trend of DPPH values. The antioxidant activities of six whole foxtail millet varieties reported by Zhang et al. (2017) were all significantly greater ($p < 0.05$) than that of the corresponding dehusked millets, and their scavenging DPPH and ABTS and FRAP values of the whole millet were 1.34–1.98 times, 2.74–4.31 times and 2.14–3.87 times, respectively, as that of the dehusked millet. For those different kinds of millet (kodo millet, figer millet, foxtail millet, proso millet, little millet, pearl millet) studied by Chandrasekara et al. (2012), it was also found that the antioxidant capacity (DPPH and hydroxyl radical scavenging activities) of whole millet sample was significantly higher ($p < 0.05$) than the corresponding dehulled millet sample.

It was indicated that different milling degree of foxtail millets should decrease antioxidant capacity by all three antioxidant assays as compared to the whole millet grain, which was also present in other cereal grains. It was reported that the cell antioxidant activity of *japonica* rice and *indica* rice decreased by more than 90 % at the milling degree of 9.60 % (Liu et al., 2015). At 10 % milling degree, the DPPH values of free and bound phenolics of black rice were decreased from 81.04 to 3.60 μmol TE/g and 13.02 to 0.59 μmol TE/g, and ABTS values were decreased from 23.57 to 3.52 μmol TE/g and 4.11 to 0.46 μmol TE/g, respectively (Paiva et al., 2014). Moreover, antioxidant capacity of rice grain after treatment with different milling processes was closely correlated with the phenolic content (Ma et al., 2020). This was also reflected in our study, the loss of antioxidant activity of free phenolics shared the similar trend with bound phenolics of foxtail millet, and displayed the maximum values in the whole millet grain. The decreases in the scavenging DPPH and $ABTS^{+}$ radical activity and FRAP mainly resulted from the loss of phenolic compounds in foxtail millet during the processes of milling treatment.

3.7. Correlation analysis

Pearson's correlation analysis is used to analyze the relationship between different variables of the foxtail millet samples with different milling degree, and the results are presented in Fig. 5. Stronger positive (red) and negative (blue) correlations display darker colors and flatter ellipses, respectively.

Food color is a key sensory attribute that can directly impact consumer preferences and purchasing decision. The b* value demonstrated a strong positive correlation ($r = 0.95$) with TCC, indicating that carotenoids were the main contributor of yellow color, and retained during the milling process. In contrast, the b* value showed significantly negative correlation with TPC ($r = -0.96$), TFC ($r = -0.92$), GABA ($r = -0.94$), demonstrating that the phenolic components and GABA were probably removed or degraded during milling process. The L* value was significantly positively correlated ($r = 0.96$) with the total starch content and significantly negatively correlated ($r = -0.88$) with total ash content, suggesting that percentage of starch increases, while the ash content associated with minerals and fiber was gradually reduced by removing from the outer layer of millet grains during milling process. The a* value displayed a significantly positive correlation ($r = 0.94$) with PA level, showing that milling process results in loss of PA. In addition, significantly positive correlations between TPC and DPPH($r = 0.96$), ABTS($r = 0.94$), FRAP($r = 0.95$), as well as TFC and DPPH($r = 0.98$), ABTS($r = 0.95$), FRAP($r = 0.97$), were observed, because phenolic compounds including flavonoids are important contributors of antioxidant activity of foxtail millets.

4. Conclusions

The various nutritional and functional compounds of foxtail millets with different milling degree are related to the retention degree of each part during milling process, for their uneven distributions in the grain seeds. With the increase of milling degree, the husk, bran and endosperm are gradually removed, the crude protein, crude fat, phytic acid, and total carotenoid contents showed similar first increasing and then decreasing trend, except for the total starch percentage with continuous increasing trend and total ash with continuous decreasing trend. And TPC, TFC, GABA content, and antioxidant capacity of foxtail millets decreased significantly ($p < 0.05$) during milling process. The thirty-two detected phenolic compounds in the whole foxtail millet were significantly ($p < 0.05$) reduced due to the different milling degree, among which seven of them was undetectable with higher milling degree, including *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, apigenin-C-dihexoside, apigenin-C-pentosyl-C-hexoside isomers in free form and vanillic acid in bound. Foxtail millets with higher milling degree display

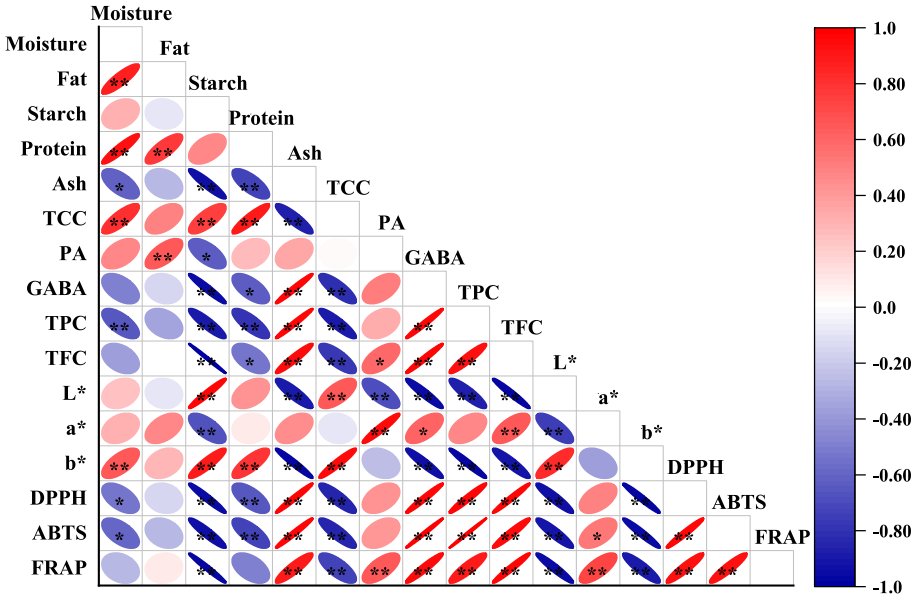

Fig. 5. The correlation analysis of different variables from the foxtail millets with different milling degree. * represents $p < 0.05$, ** represents $p < 0.01$.

relatively lower nutritional and healthy quality. By comprehensively considering the retention of nutrients and functional components, as well as the achievement of removing the grains' outer layer, the two-time milling foxtail millet is suggested to be selected as the appropriate milling degree for consumption. The research results might provide important basis for improving processing technology and maintaining edible and functional properties of foxtail millet, and indicate the importance to choose the appropriate milling degree to reduce the loss of nutrients and bioactive compounds.

CRediT authorship contribution statement

Youyang Zhang: Writing – original draft, Methodology, Investigation, Formal analysis. **Jiapeng Jiao:** Writing – review & editing, Methodology, Investigation, Data curation. **Meng Li:** Writing – review & editing, Methodology, Investigation. **Zhenchuan Wei:** Resources, Investigation. **Xiangxiang He:** Investigation, Formal analysis. **Daniela D. Herrera-Balandrano:** Writing – review & editing, Conceptualization. **Jinle Xiang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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