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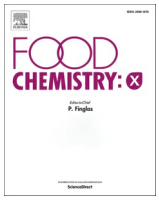

Nutrient and metabolite characteristics of the husk, bran and millet isolated from the foxtail millet (*Setaria italica* L.) during polishing

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ABSTRACT

The utilization of byproducts from foxtail millet polishing can reduce food loss and waste. Thus, it is necessary to know the chemical compounds from the millet and the segregation of the layers. The nutrients including minerals were compared among the husk, bran, and millet, and a LC-MS metabolomics analysis was also performed among them. The results showed that the protein, crude fat and 4 fatty acids, seven minerals, the nitrogen-containing compounds and phenolic acids were at much higher levels in the bran part than the husk and millet, whereas the husk only contained higher levels of dietary fibre, and some minerals. The millet section, as the edible part, contained the lowest level of chemical constituents. It illustrated that the bran part contained more functional and nutritional components than the millet and husk part. Therefore, the bran of the foxtail millet should be a food resources instead of wasting.

1. Introduction

Reducing loss and waste in agricultural products has been a significant trend in recent years. Unfortunately, food loss and waste are still a serious problem. On the one hand, one-third of all food has been lost from its production to our dinner table; on the other hand, above 800 million people worldwide have not enough access to food (Mokrane et al., 2023). Therefore, much more needs to be done to diminish food loss and waste.

The whole grain consumption can effectively diminish the food loss and waste because it maintains the bran part against the refined grain when the grain milled or polished. In fact, the whole grain is also called the naked cereal seed (caryopsis), and it contains the starchy endosperm, germ, and bran of the caryopsis except the hull part (Frølich et al., 2013). However, in traditional grain milling, only the starchy endosperm part is reserved, and the husk, germ and bran part has been discarded.

It is reported that the ingestion of husk- or bran-contained food can greatly affect the human's health because it can decrease the risk of type II diabetes mellitus, cardiovascular disease, obesity, and intestine problem (Jiménez-Pulido et al., 2024; Wu et al., 2015). The reason is that the husk and bran contain large amounts of fibre, such as β-glucan, arabinoxylan, cellulose, which can provide satiety and accelerate food residue transport in the colon (Stevenson et al., 2012). Otherwise, minerals, vitamins, phytic acid, phenols and other beneficial components are also rich in the husk and bran (Beloshapka et al., 2016; Stevenson et al., 2012). Thus, it is necessary to retain the husk or bran part for food material from the aspect of human health. However, there are not enough data for their utilization, and a comprehensively analysis of the husk, bran and refined grain is necessary.

In China, the porridge made from the foxtail millet are widely consumed due to its nutrition and delicious. The foxtail millet (*Setaria italica* L.), originated from China, is a diploid gramineous crop. It is widely planted in northern China due to its adaption on the arid and semi-arid areas (Wang et al., 2023). The foxtail millet seed contains three parts: the outer layer of the seed is the husk, the middle layer is the

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bran, and the inner part is the edible part (millet) (Wang et al., 2023). After harvest, the foxtail millet needs to be polished to provide good palatability before cooking, and the byproducts of husk and bran are removed during the millet polishing (Zhang et al., 2021). However, previous studies show that the husk and bran contain high level of flavonoids and phenolic acids, and the content of them are greatly higher than the millet (Ding et al., 2019). The flavonoids mainly contain the glycosides of apigenin, kaempferol, and luteolin, whereas the phenolic acids mainly contain the ferulic acid, chlorogenic acid, *p*-coumaric acid, and syringic acid (Zhang et al., 2021). Meanwhile, the bran of foxtail millet also contains high level of oils. It means the husk and bran part may contain more nutrients and functional compounds than the millet.

To comprehensively utilize the whole grain of the foxtail millet, it is necessary to investigate the components in the husk, bran, and millet. Thus, a comparison has been conducted to analyze the chemical differences in the three parts of foxtail millet by nutrition analysis and metabolomics.

2. Materials and methods

2.1. Chemicals

LC-MS-grade methanol was purchased from Fisher (Pittsburgh, USA). Formic acid was purchased from TCI (Tokyo, Japan). The mixture of 37 fatty acid methyl esters was purchased from ANPEL laboratory technologies Inc. (Shanghai, China). The glucose, copper sulfate ($CuSO_4$), potassium sulphate (K_2SO_4), sodium hydroxide (NaOH), and boric acid (H_2BO_3) were purchased from Energy Chemical Inc. (Shanghai, China). α-Amylase and trypsin were purchased from Sigma (St. Louis, MO, USA). Petroleum ether (30–60 °C) and nitric acid were purchased from Yongda Chemical Reagent Co., Ltd. (Tianjin, China). The mineral standards were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Milli-Q water was used throughout this study (Millipore, Billerica, MA, USA).

2.2. Instruments and apparatus

A gas chromatography (GC) was used for fatty acid detection (7820 A, Agilent Technologies, Santa Clara, CA, USA). The GC is coupled with a flame ionization detector (FID) and an auto-sampler, and a HP-88 column (100 m × 0.25 mm i.d., 0.20-μm film thickness, Agilent Technologies, Santa Clara, CA, USA) was used for volatile separation. An ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry was used for metabolomics analysis (UPLC, ExionLC; Q-TOF, X500R; AB Sciex company, Massachusetts, USA). The binary pump UPLC system can tolerate 100 MPa pressure and is with an auto-sampler. An electrospray ionization source is coupled with the Q-TOF section. A Zorbax Eclipse Plus C18 column was used for the metabolite separation (150 × 2.1 mm i.d., 1.8 μm particle size, Agilent Technologies, Little Falls, DE, USA), and a SCIEX OS software was used for data acquisition. An inductively coupled plasma mass spectrometry was used to detect the minerals (ICP-MS, PQMS Elite, Analytikjena company, Germany). The microwave digestion apparatus was used to obtain the mineral solution (Ultraclave V, Milestone company, Italy). The vacuum freeze-drying equipment was purchased from Martin Christ (D37520, Osterode, Lower Saxony, Germany). A digestion furnace (KDN-20C, Hangzhou Lübo instrument Co., Ltd., Hangzhou, China) was used for sample digestion and an automatic Kieldahl apparatus (KDN-520, Hangzhou Lübo instrument Co., Ltd., Hangzhou, China) was used for protein detection. The centrifuge was purchased from Thermo Scientific (3SR+, CA, USA).

2.3. Sample preparation

The foxtail millet of T7, Zhonggu 2, Jigu 42, Jigu 18, Jigu 19, Yugu 18, Baimi 1, Jichuang 1, Huihuajinmi, and Jigu 37 were harvested and sun-dried (10%–12% moisture content) in our experimental station (Gaocheng district, Shijiazhuang City, Hebei, China). About 100 g of each sample were dehulled (rice mill, SY88-TH, Ssang Yong Machinery industry co. Ltd., Incheon, Korea) for 5 times and the husk (about 15%), bran (about 20%) and millet (about 65%) were collected. Then the samples were freeze-dried to obtain an equal low-moisture material. The samples were milled into powders with a pulverizer (IKA, Staufen, Germany) and sieved with 80 mesh for experimental use.

2.4. The nutrient detection for the husk, bran, and millet

The total lipid was determined by Soxhlet extraction with our previous method (Li et al., 2021). Briefly, 1 g sample was filled in a filter paper bag and extracted by the petroleum ether at 50 °C for 5 h. The lost weight of the sample was the total lipid content.

The protein was detected by the Kjeldahl method with some modifications (Wiedemair et al., 2019). Briefly, 0.5 g sample was mixed with 8 mL H_2SO_4, 0.4 g $CuSO_4$ and 6.0 g K_2SO_4, and digested for 2.5 h at 420 °C until the digestion liquid was transparent. The mixture was distilled with adding 50 mL water and 10 mL 40% (*w/v*) NaOH solution using an automatic Kieldahl apparatus. A 10-mL boric acid (5%, *v/w*) was used to receive the NH_3, and the H_2SO_4 solution (0.0223 mol/L) was used to calculate the protein content.

The total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were detected by the previous study with some modifications (Dong et al., 2019). Briefly, 1 g sample was defatted by 20 mL petroleum ether twice in a 50 mL centrifuge tube. Then, the sample was hydrolyzed by the α-amylase in 10 mL phosphate buffer solution (0.08 mol/L, pH 6.0, 95 °C) for 30 min. Afterwards, the pH of the solution was adjusted to 4.5 ± 0.2 with 0.01 mol/L HCl, and the mixture was continually hydrolyzed by acid protease at 60 °C for 30 min. The hydrolysate was centrifuged at 2907 ×*g* for 10 min, and the supernatant was collected to a flask. Then, 10 mL water (70 °C) was used to wash the residue twice, and the supernatant was also collected. The supernatant was used to detect the SDF and the residue was used to detect the IDF.

To detect the IDF, 15 mL 78% ethanol (*v*/v) was used to wash the residue twice, and another 15 mL 95% ethanol (v/v) was also used to wash the residue twice. Then, 15 mL acetone was used to wash the residue twice, and the residue was weighed after dried in an oven at 105 °C. To detect the SDF, four-fold volume ethanol (95%, v/v, 60 °C) was added to the supernatant above, and the precipitate was centrifuged after one hour. The residue was the SDF and detected according to the IDF procedure. The TDF was calculated as the IDF plus the SDF.

2.5. The fatty acid detection using GC-FID

The fatty acid components were detected according to the description of a previous study with slight modifications (Ivanova-Petropulos et al., 2015; Li et al., 2021). Briefly, 0.1 g millet powder was mixed with 0.1 g pyrogallic acid, 2 mL ethanol (95%, *v*/v), 4 mL water, and 10 mL hydrochloric acid (8.3 mol/L) in a 50 mL centrifuge tube. Then the mixture was heated in an 80 °C water bath for 40 min for lipid hydrolysis. The hydrolysate was diluted with 10 mL ethanol (95%, v/v), and extracted for 3 times with 10 mL petroleum ether. The combined extract was dried at 80 °C, and the residues were re-dissolved in an 8-mL sodium hydroxide-methanol solution (2%, *w/v*) and heated for 15 min. Finally, 7 mL of 14% BF3-methanol solution was added and continually heated for 2 min. The derivatization reaction was terminated by placing it on ice, and the fatty acid methyl esters were extracted using 2 mL n-hexane.

Fatty acid methyl esters were analyzed using the GC-FID. The injection port temperature and FID temperature were 240 °C, respectively. The injection volume was 1 μL with splitless mode. The initial temperature of the column was 130 °C, and then increased to 240 °C at a rate of 4 °C/min and held for 20 min. Nitrogen (purity >99.999%) was used as the carrier gas with the flow rate of 1.5 mL/min. The air flow and hydrogen flow were 400 and 30 mL/min, respectively. Four standard

curves were established to quantify the content of the fatty acids with $R^2 > 0.99$ and low limit of detection and quantitation (Supporting information, Table S1).

2.6. Nontargeted metabolomics analysis of the husk, bran, and millet

Sample extraction was performed according to a previous study (Li et al., 2018). Briefly, 0.1 g sample were mixed with 5 mL methanol-water solution (70%, *v*/v) in a 15 mL centrifuge tube, and the tubes were water-bathed at 70 °C for 30 min. Then the infusion was filtered through a 0.22 μm membrane into a glass-vial for LC-MS analysis. All the samples were prepared in triplicate. A quality control (QC) sample was prepared by mixing 100 μL of each sample together and were used to evaluate the stability and reproducibility of the metabolomics analysis. Briefly, the mobile phases of UPLC with solvent A (water containing 0.1% formic acid, v/v) and solvent B (methanol) were performed at a flow rate of 0.4 mL/min as follows: 0–4 min, 10%–15% B; 7–9 min, 25%–32% B; 16–22 min, 40%–55% B; 28–30 min, 95% B; 31–35 min, 10% B. The injection volume was 10 μL. Electrospray ionization (ESI) in positive ionization mode and IDA (Information Dependent Acquisition) method were performed with the following parameters: ion source gas 1 and 2 were 50 psi, respectively. The CAD gas was set 7 psi. The spray voltage was 5500 V. The collision energy and declustering voltage was 10 and 70 V, respectively. The mass scan range was set 100–1000 Da.

2.7. The mineral detection using ICP-MS

The minerals were detected according to a previous study with some modifications (Nardi et al., 2008). Briefly, 0.1 g sample was mixed with 5 mL HNO_3. The digestion temperature was tested from 110 °C up to 220 °C with the microwave power of 1200 W. The digestion solution was eluted to 100 mL and analyzed by the ICP-MS. A total of 16 standard curves were established to quantify the content of the minerals with $R^2 > 0.99$ and low limit of detection and quantitation (Supporting information, Table S2).

2.8. Data treatment and statistical analysis

The peak extraction and alignment were performed using the MarkerView™ software (AB Sciex company, Massachusetts, USA). The principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) were conducted with Simca-P software (version 11.5, Umetrics AB, Umeå, Sweden) after Pareto-scaling. A cluster analysis was performed with a Pearson correlation after auto-scaling using the MultiExperiment Viewer 4.9.0 software. Student's *t*-test was employed to analyze the significant difference using the SPSS 21.0 software (IBM, USA).

3. Results and discussion

3.1. The nutrient differences in the husk, bran, and millet

The nutrients from the husk, bran and millet of ten foxtail millet cultivars were compared. The bran part contained the highest level of the fat due to it contains the germ of the foxtail millet, which can reach 15.96 ± 2.37 g/100 g. In contrast, the crude fat content in husk and millet was 0.62 ± 0.44 g/100 g and 3.57 ± 0.98 g/100 g, respectively (Fig. 1a). It showed that the millet bran had a similar level of the crude fat to the wheat bran (11%–18%) and lower than the rice bran (17.3%–27.4%)(Goffman et al., 2003; Kumari et al., 2017). Furthermore, the main unsaturated fatty acids (oleic acid and linoleic acid) were also rich in the bran, which were 73.3-fold and 12.4-fold higher than those in husk and millet. In addition, the saturated fatty acids (palmitic acid and stearic acid) were 14.0-fold and 7.6-fold higher than the husk and millet, respectively (Fig. 1d). The dominant fatty acid was the linoleic acid and occupied 53.6% of the total fatty acid in the bran and reached 10.37 ± 1.72 g/100 g. Both the proportion and the content of the linoleic acid from the millet bran is higher than the rice bran, which means the millet bran can be an excellent resource for polyunsaturated fatty acid (Goffman et al., 2003). Otherwise, the bran oil also contained lots of tocopherol, tocotrienol, and oryzanol, and they possessed many bio-functions that have been proven effectively against reactive oxygen species and for preventing chronic diseases (Yin et al., 2022).

The protein was also at the highest level in the bran part, and the husk part contained the lowest level. The protein was 12.04 ± 1.02 g/100 g in millet, and was similar with the previous study (12.48 ± 0.41 g/100 g) (Liang et al., 2009). It was almost equal to the wheat flour (Ma et al., 2007) and at higher levels than the rice (Sohn et al., 2004) and maize (Abiose & Victor, 2014). Moreover, the level of the bran reached to 20.62 ± 1.72 g/100 g and was 1.71-fold and 4.44-fold higher than the

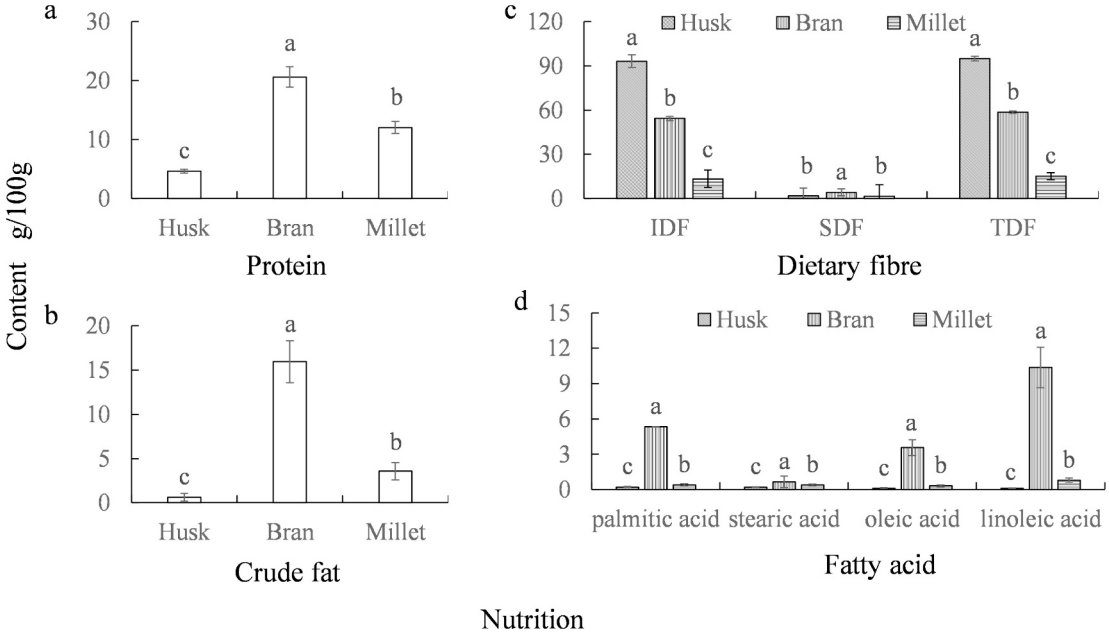

Fig. 1. Nutrient differences in the husk, bran, and millet of the foxtail millet.

millet and husk, respectively (Fig. 1c). It was also higher than the protein in the wheat bran (15%–18%) (Brier et al., 2015; Noort et al., 2010). It meant that the whole grain of the foxtail millet (including the bran part and millet part) was an excellent resource of protein.

The dietary fibre (DF) contains the SDF and IDF. The IDF mainly consists of cellulose, hemicellulose, lignin, and parts of the arabinoxylan, whereas the SDF mainly consists of oligosaccharide, β-glucan, pectic substances and hydrocolloids (Cheng et al., 2021; Kshirsagar et al., 2020). As reported, the DF possesses the functions of anti-obesity, anti-cancer, and reduce the risk of cardiovascular disease and type II diabetes (Cheng et al., 2021). The foxtail millet is rich of the DF (Fig. 1b). The TDF could reach 95% of the whole husk weight, and most of them was the IDF (93.18 ± 4.8 g/100 g). It seemed that the husk contained little nutrients but large of the IDF, which demonstrated that the husk could not provide diverse nutrients when consumed by people. However, the bran part was also rich in the TDF, and the IDF level was less than the husk while the SDF was significantly higher than the husk. The TDF, IDF, and SDF of the millet bran were 58.54 ± 7.75, 54.30 ± 5.33, 4.24 ± 2.42 g/100 g, respectively. All of them are higher than the wheat bran and rice bran (Neeta et al., 2018; Stevenson et al., 2012). The high level of SDF means the bran may contain large of oligosaccharide. Neither the SDF nor the IDF were rich in the millet and the TDF, IDF, and SDF only were 15.09 ± 2.34, 13.44 ± 1.48, 1.66 ± 0.85 g/100 g, respectively. Though the content of the DF of millet were lower than the husk and bran, they were higher than the rice and wheat flour (Kiin-Kabari & Giami, 2015; Lovegrove et al., 2019). It suggested that the millet part could provide high levels of DF as a kind of minor grain.

3.2. The metabolite differences in the husk, bran, and millet

To comprehensively understand the metabolite differences among the husk, bran, and millet, a non-targeted metabolomics analysis using UPLC-QTOF/MS was performed to detect the metabolites in 10 foxtail millet cultivars. After the peak alignment and removal of interfering ions (detected in the blank control sample), a total of 4796 metabolite ions was obtained for statistical analysis. Among them, the relative standard deviations of 4158 metabolite ions were <30% in five QC samples in which total peak areas reached 77.8%. In addition, the QC samples were in the center of the PCA score plot (Fig. 2a). These results indicated excellent reproducibility and stability of the metabolomics analysis. The unsupervised principal component analysis (PCA) clearly divided the husk, bran, and millet of the foxtail millet into three groups (Fig. 2a). The husk group was in the left part of the PCA score plot, while the bran group and millet group were in the middle and right part of the PCA score plot, respectively. It indicated there were different metabolites among the husk, bran, and millet parts. A supervised partial least square discriminant analysis (PLS-DA) was then performed to discover the different metabolites (Fig. 2b). The model was cross validated without overfitting (Fig. 2c). The loading plot of the PLS-DA model was used to screen the main different metabolites among the 3 groups (Fig. 2d).

Twenty-nine compounds, which mainly contained 8 nitrogen-containing compounds, 11 phenolic acids and 10 flavonoids were tentatively identified (Table 1). These compounds were identified based on the comparison of retention time, accurate mass, tandem MS spectra, the HMDB database and previous references (Zhang et al., 2021). The amount of the phenolic acids and flavonoid were great larger than the previous reports (Zhang et al., 2017; Zhang & Liu, 2015), which provide the knowledge for further investigate the phenols from foxtail millet.

A heatmap was applied to show the content of different metabolites in the husk, bran, and millet. Blue indicates that the metabolite level was less than the mean level, whereas orange indicates that the metabolite level was higher than the mean level (Fig. 3). The samples were clustered into the husks, brans, and millets, which was consistent with the PCA result (Fig. 2a). The metabolites were also clustered into two groups. Group A mainly contained the nitrogen-containing compounds, and phenolic acids, which were apparently higher in brans. It meant that the vitamins (pantothenic acid and folinic acid), amino acids (tyramine and glutamic acid), phenolic acids (p-coumaric acid isomer 1 and 2, 4-p-coumaroylquinic acid, 5-O-feruloylquinic acid, caffeic acid 3-O-glucuronide, caffeic acid 3-O-glucuronide-methyl-caffeic acid, and 2-hydroxycinnamic acid) were rich in the brans. In contrast, group B mainly contained the flavonoids, and rich in the husk group. The results were consistent with previous studies that the total free phenolic contents were higher in brans than those in husks and millets (Zhang et al., 2021).

To compare the different compounds among the three parts, a *t*-test was performed on the compounds in Table 1. Seven phenolic acids in the bran showed significantly higher than those in the husk and millet, while only four phenolic acids showed higher content in the husk than in the

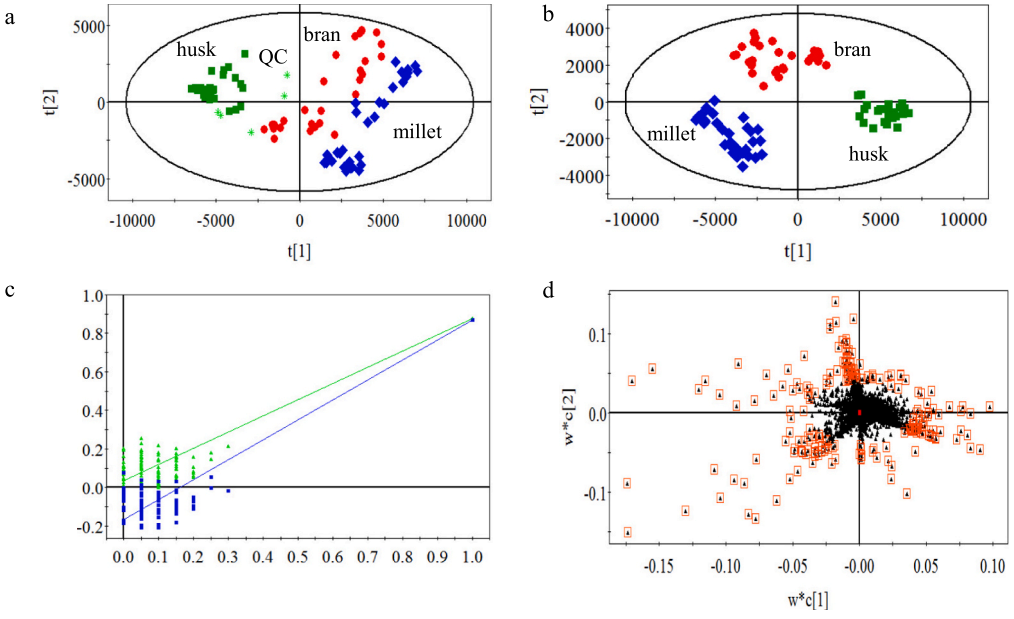

Fig. 2. Multivariate statistical analysis of husk (squares), bran (circles) and millet (diamonds): (a) PCA score plot; (b) PLS-DA score plot, R2X = 0.405, R2Y = 0.091, Q2 = 0.873; (c) Cross-validation plot of the PLS-DA model with 200 permutation tests (intercepts of R2 and Q2 were 0.0342 and − 0.173, respectively); (d) PLS-DA loading plot (black triangles with boxes represent most different metabolites).

Table 1
Putative structures of the compounds discovered in husk, bran and millet.

RT/min	Compound name	Detected *m/z* (ESI+)	Theoretical m/z (ESI+)	Mass error (ppm)	MS/MS fragments	Ion type
0.83	Glutamic acid	148.0605	148.0604	0.68	102, **84**, 56	M + H
1.38	2-Phenylacetamide	136.0755	136.0757	−1.47	118, **91**	M + H
1.38	2-Hydroxycinnamic acid	165.0543	165.0546	−1.82	95, 91, 77	M + H
1.55	Adenosine	268.1031	268.104	−3.36	**136,** 119	M + H
1.72	Tyramine	160.0734	160.0733	0.62	130, 117, **115**, 72	M + Na
4.07	Pantothenic acid	220.1182	220.1179	1.36	**202**, 124, 72	M + H
5.72	Folinic acid	474.1720	474.1732	−2.53	345, **327**, 284, 259	M + H
6.61	Trigonelline	138.0547	138.055	−2.17	108, **78**	M + H
6.74	p-Coumaric acid isomer 1	147.0444	147.0446	−1.36	119, **91**	M + H-H_2O
9.66	p-Coumaric acid isomer 2	147.0443	147.0446	−2.04	119, **91**, 65	M + H-H_2O
9.66	4-p-Coumaroylquinic acid	339.1079	339.108	−0.29	**147**, 119, 91	M + H
10.37	5-O-Feruloylquinic acid	369.1170	369.118	−2.71	**177**, 145, 117, 91	M + H
10.75	p-Coumaric acid isomer 3	147.0446	147.0446	0.00	119, **91**	M + H-H_2O
11.53	Apigenin 6-C-arabinoside 8-C-glucoside	565.1540	565.1552	−2.12	547, 469, **379**, 337	M + H
11.95	Luteolin C-glucoside	449.1072	449.1078	−1.34	431, 413, **329**, 299	M + H
12.21	4-Hydroxy-3-methoxycinnamaldehyde	179.0702	179.0703	−0.56	147, 119, **91**, 79	M + H
12.46	Orientin-O-glucoside	611.1615	611.1607	1.31	449, 329, **287**	M + H
13.52	Isovitexin 2”-O-glucoside	595.1665	595.1657	1.34	**433**, 313, 271	M + H
13.96	Apigenin 6-C-glucoside 8-C-arabinoside	565.1535	565.1552	−3.01	547, 469, **379**, 337	M + H
14.31	Kaempferol 3-O-glucoside	449.1088	449.1084	0.89	**287**	M + H
16.7	Apigenin 4’-O-glucoside	433.1123	433.1129	−1.39	**271**	M + H
17.41	Caffeic acid 3-O-glucuronide	357.0811	357.0816	−1.40	**181**, 153, 114	M + H
17.41	Caffeic acid 3-O-glucuronide-methyl-caffeic acid	553.1528	553.1552	−4.34	357, 227, **181**	M + H
17.48	7-O-Methylluteolin-glucoside	463.1219	463.1235	−3.45	301	M + H
23.92	p-Coumaric acid isomer 4	147.0446	147.0446	0.00	119, **91**	M + H-H_2O
24.24	Malvidin	331.0806	331.082	−4.23	331, **315**, 287, 98	M + H
26.11	Malvidin arabinoside	445.1124	445.1129	−1.12	445, **331**, 315, 147	M + H
26.26	4-Oxoisotretinoin	315.1971	315.1961	3.17	**297**, 147, 123	M + H
27.39	Phthalic acid	149.0238	149.0239	−0.67	121, 93, **85**, 65	M + H-H_2O

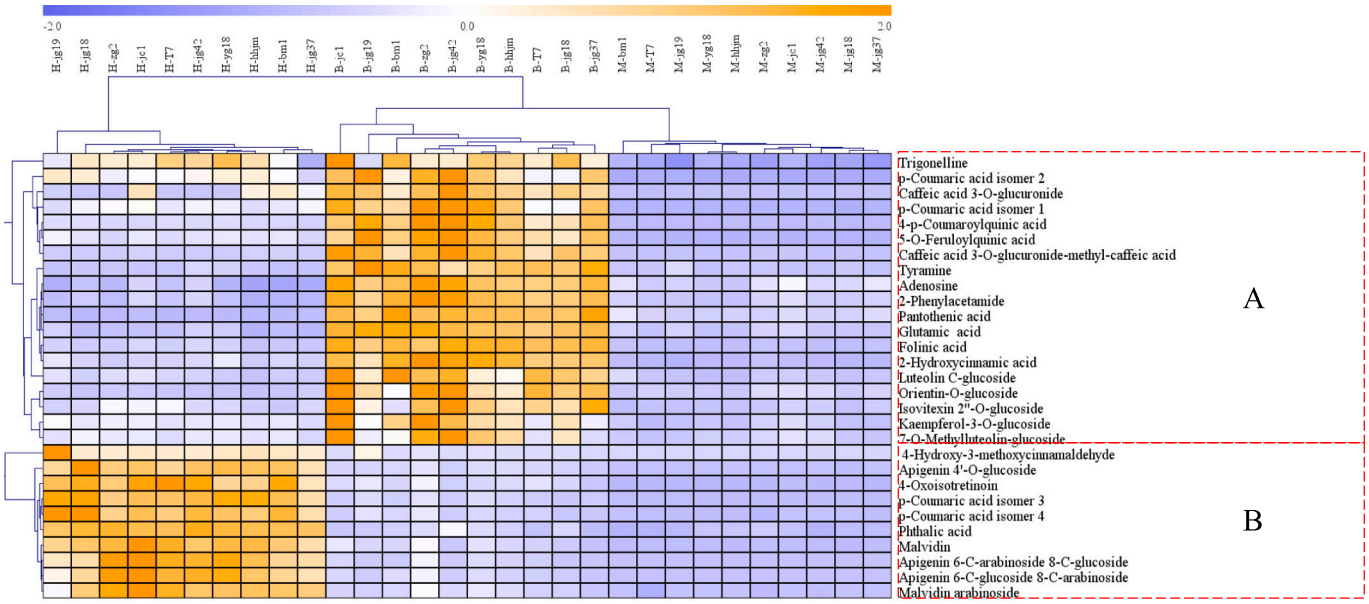

Fig. 3. A heatmap showing the distribution of the metabolites among the husk, bran, and millet. The data are auto scaled and clustered according to the Pearson correlation coefficients. Group A mainly contains phenolic acids, nitrogen-containing compounds, and several flavonoids, whereas group B mainly contains flavonoids.

bran and millet ($p < 0.05$, see Supporting information, Fig. S1). In contrast, five flavonoids were at significantly higher levels in the bran, and five flavonoids were also at significantly higher levels in the husk ($p < 0.05$, see Supporting information, Fig. S2). The results showed that the millet contained the lowest level of phenolics. Phenolics shows high activities in antioxidant, antitumor, and reduce the risk of cardiovascular diseases, diabetes and stroke (Li et al., 2020). The higher level of phenolics of the husk and bran meant that they had higher bioactivities than the millet. It was agreed to the previous studies that the bran showed higher antioxidant because of the higher phenolics (Kim et al., 2006). Otherwise, the nitrogen-containing compounds were also at higher levels in the bran, and they mainly contained the amino acids and vitamins ($p < 0.05$, see Supporting information, Fig. S3). In other words, the bran was rich of the functional compounds.

3.3. *The mineral differences in the husk, bran, and millet*

Minerals are important substances that make up the body's tissues or component for enzymes. A total of 16 minerals had been detected from the husk, bran, and millet using the ICP-MS. The multivariate statistical

analysis showed there were great differences in the contents of the minerals among the three compositions of the foxtail millet (Fig. 4a). Seven minerals (Na, K, Cr, Al, V, Ca, Sr) were at higher levels in the husk, and seven minerals (Mn, Fe, Cu, Mg, Zn, Mo, Sn) were at higher levels in the bran (Fig. 5). It was noteworthy that 4 trace elements (Mo, V, Sr, and Se) have been rarely reported in the cereals, which has a potential benefit for the human body.

The bran part contained large content of molybdenum (Mo) and was 5.38-fold and 6.14-fold higher than the millet and husk, respectively. Though trace of Mo is needed by humans, it is the composition of many enzymes and relates with the skeletal and tooth development, immune system, and cardiovascular (Chan et al., 1998).

In the husk part, there were also high level of vanadium (V) and the content reached 0.24 ± 0.05 mg/kg. This element has the functions of reduce the risk of cardiovascular disease as well as play a key role in the metabolism of the thyroid and of iron (Gruzewska et al., 2014).

In addition, the strontium (Sr) was also rich in the husk. It has been proved to reduce the risk of vertebral fractures in postmenopausal women with osteoporosis (Usuda et al., 2007).

The Se is a kind essential element for humans, and its deficiency may cause cancer, cardiovascular disease, diabetes and 40 other diseases (Liu et al., 2016). It was showed that there were no significant differences among the husk, bran and millet, and the content was about 200 μg/kg, which was similar to the previous reports (Liang et al., 2020). The content of Se in foxtail millet was greatly higher than that in the selenium-enriched rice of which the Se content was 40–300 μg/kg (Chinese Standard: GB/T 22499–2008), and it meant that the foxtail millet was a kind of selenium-enriched cereal.

The husk and bran of the foxtail millet are rich of minerals, which results in the millet minerals decrease with the polishing of the foxtail millet.

Overall, it confirmed that the husk did not have enough nutritional value due to its main constitution was fibres. Even the phenolic acids and flavonoids does not need to be concerned because they are at low levels (about 1% content) without special varieties (Zhang et al., 2021). What should be concerned about is the protein, lipid, vitamin, mineral and the dietary fibre (especially the SDF) from the brans. Moreover, the foxtail millet contains low level of antinutritional factors (about 0.2% tannins and 0.4% phytate) and gluten (Rana et al., 2023; Sharma et al., 2015), it means the foxtail millet is easily to digest and would not result in allergy. Thus, the utilization of the whole grain of foxtail millet should be paid attention.

4. Conclusions

These results support the utilization of cereal byproducts and the whole grain consumption. The findings show that the bran of the foxtail millet contained much higher levels of protein, crude fat, unsaturated fatty acid, dietary fibre, nitrogen-containing compounds, and phenolic acids, and minerals than the husk and millet part. The millet, as the edible part of the foxtail millet, only is rich in the carbohydrate and the husk only contains lots of fibre. It suggests that the bran of the foxtail millet should be a food resources instead of wasting. Thus, the whole-grain food of foxtail millet including sprouted foxtail millet or the bioactive constituents from its bran part should be paid more attention in further studies.

CRediT authorship contribution statement

Pengliang Li: Writing – original draft, Methodology, Funding acquisition. **Xinru Cai:** Methodology. **Shaohui Li:** Methodology. **Wei Zhao:** Project administration. **Junli Liu:** Methodology. **Xiaodi Zhang:** Methodology. **Aixia Zhang:** Funding acquisition. **Linlin Guo:** Software. **Zengning Li:** Funding acquisition. **Jingke Liu:** Funding acquisition.

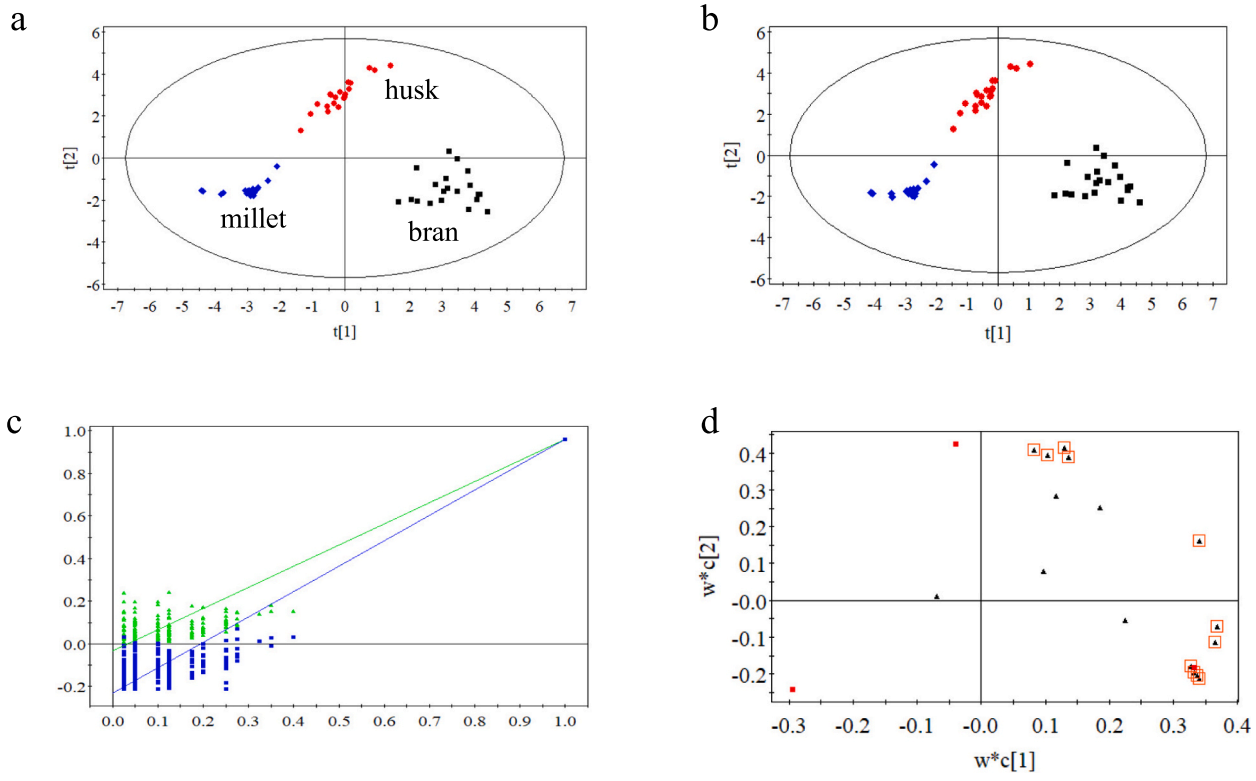

Fig. 4. Multivariate statistical of the minerals in husk (circles), bran (squares) and millet (diamonds): (a) PCA score plot; (b) PLS-DA score plot, R2X = 0.446, R2Y = 0.316, Q2 = 0.926; (c) Cross-validation plot of the PLS-DA model with 100 permutation tests; (d) PLS-DA loading plot (black triangles with boxes represent most different minerals).

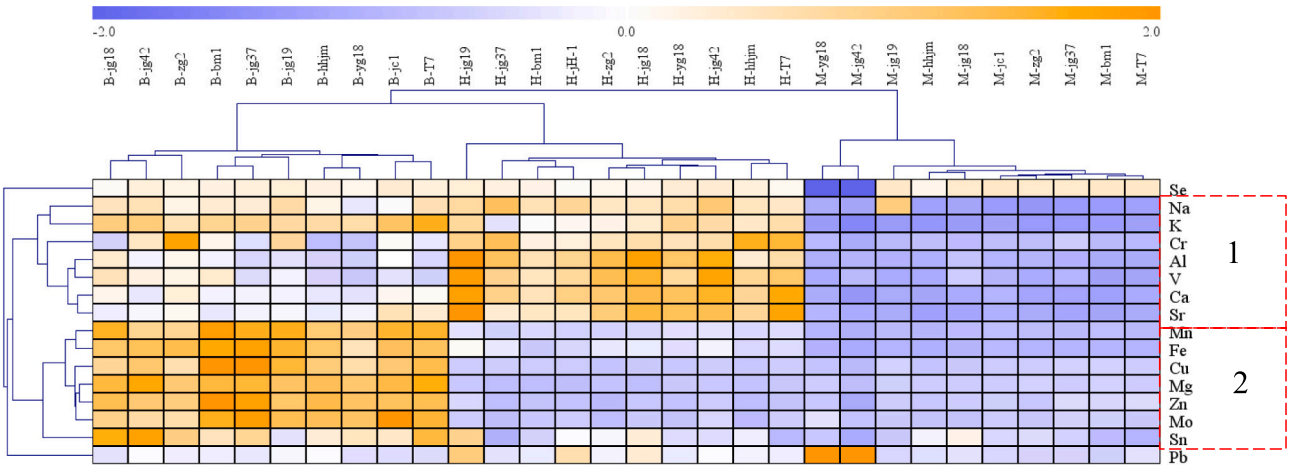

Fig. 5. A heatmap showing the distribution of the minerals among the husk, bran, and millet of the foxtail millet.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101541.

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