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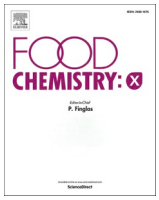

Identification of the chemical composition of distiller's grain polyphenols and their effects on the fecal microbial community structure

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ABSTRACT

Distiller grains are the main by-products of *Baijiu* production and are usually discarded, ignoring their abundant functional phytochemicals. The free and bound polyphenols from distiller grains were extracted and their potential effect on modulating fecal microbiota was investigated using *in vitro* fecal fermentation. The results showed that 34 polyphenols were quantified from distiller grains. The antioxidant activity was positively correlated with quercetin, myricetin, epicatechin, and naringenin. The abundance of *Bifidobacterium*, *Ruminobacterium*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria was enhanced by distiller's grain polyphenols by approximately 10.66-, 6.39-, 7.83-, 2.59-, and 7.74-fold, respectively. Moreover, the production of short-chain fatty acids (SCFAs), especially acetic, butyric, and propionic acid, was promoted (increased 1.99-, 1.71-, and 1.34-fold, respectively). Correlated analysis revealed quercetin, daidzein, and kaempferol as the key polyphenols by analyzing the effects on gut microbiota and SCFAs. This study could provide a reference for converting distiller grains into high-nutrient functional food ingredients and feeds.

Introduction

Distiller's grains, which are the primary by-products of liquor production, have an annual output of up to 20 million tons, making them a significant resource in terms of by-products. In addition to containing protein, starch, and dietary fiber, they are also rich in bioactive substances such as polyphenols and peptides, which have potential research and application value (Liu et al., 2022). Previous studies have shown that distiller's grains are rich in polyphenolic compounds and that the brewing microorganisms ferment the polyphenols contained in the brewing raw materials to promote the release of bound or macromolecular polyphenols into free or small-molecular polyphenols. For instance, the long-time fermentation process specific to *Jiang*-flavor liquor triggers the formation of a high amount of small-molecular polyphenols in the brewing system. This leads to a substantial enrichment of small-molecular polyphenol compounds in *Jiang*-flavor distiller's grains (Wang et al., 2019; Liu et al., 2022). The polyphenol content of *Jiang*-flavor distiller grains (25.18 mg/g) is higher than that of *Nong*-flavor distiller grains (6.80 mg/g) and *Jianxiang*-flavor distiller grains (6.27 mg/g) (Wang et al., 2019; Yang et al., 2021). However, assuming an annual production of 20 million tons of distiller's grains, it is estimated that these grains can potentially yield 150,000–500,000 tons of polyphenol extracts annually. This abundant and cost-effective supply of polyphenols from distiller's grains holds significant research value. While, the main issue is that distiller's grains are mostly discarded as waste, resulting in no economic benefits and causing environmental pollution. Current studies primarily evaluate the antioxidant activity of polyphenols found in distiller's grains, neglecting their potential functions.

Polyphenols are active substances widely found in plants and have essential roles as antioxidants, oxidative stress regulators, and gut microbiota structure modulators (Del Campo et al., 2023; Videla & Valenzuela, 2022; Tang et al., 2021). For example, the structure of hydroxytyrosol's polyphenols confers a powerful antioxidant capacity with free radical scavenging activity, free radical chain-breaking potential, and chelation of metal ions (Del Campo et al., 2023). Polyphenols protect the tissues and cells of the body from oxidative stress-induced damage through various mechanisms, including iron chelation, enhancement of superoxide dismutase and catalase activities, prevention of liver steatosis and lipid metabolism-related changes, and modulation of oxidative stress-related signaling (Videla & Valenzuela, 2022; Del Campo et al., 2023). Furthermore, it could potentially

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improve intestinal function, such as the intake of polyphenol-rich sorghum, wheat, and corn, which can maintain the body's intestinal health by regulating the intestinal microbiota and producing beneficial metabolites (SCFAs, bile acids) (Gong et al., 2018). Polyphenolic compounds, such as quercetin, chlorogenic acid, anthocyanins, and flavan-3 alcohols, positively affect intestinal health. They can stimulate the growth of *Lactobacillus*, *Bifidobacterium*, and butyrate-producing bacteria while inhibiting the growth of *Escherichia coli*, *Enterococcus*, and *Listeria monocytogenes* (Liu et al., 2020). Therefore, some by-products of traditional fermented foods still rich in polyphenolic compounds have potential regulatory functions on the intestinal microbiota, which are valuable for research and application.

The gut microbiota is a group of symbiotic microorganisms, that play an important role in human health. It maintains the organism's normal physiological functions by defending against pathogens, and producing metabolites (Li et al., 2023). In addition, the gut microbiota catabolizes indigestible carbohydrates to produce SCFAs, which can provide energy for intestinal cells, promote food digestion and absorption, and maintain gut microbiota balance (Tang et al., 2021). Diet affects the structure of the gut microbiota and the production of SCFAs. A high intake of digestible sugars, fats, and proteins decreases the abundance of *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria and reduces the production of SCFAs (Ahmad et al., 2020). However, the intake of grains rich in active substances such as polyphenols stimulates the growth of beneficial bacteria and promotes the production of SCFAs (Chan et al., 2023). Thus, the by-products of traditional fermented foods, such as distiller's grains, which are rich in polyphenols, could improve the body's health. The function of polyphenols in improving gut microbiota to maintain overall health provides a pathway for realizing the high-value utilization of distiller's grains.

Therefore, this study identified polyphenolic compounds from distiller's grains, explored their antioxidant activity, potential for modulating the structure of fecal microbial communities, and effects on SCFAs, and resolved key polyphenolic compounds. The objective of this study was to provide guidance for efficient reuse and establish a scientific basis for achieving high-value utilization of distiller's grains.

Materials and methods

Materials

A total of 15 distiller grain samples were collected from three famous companies (Guizhou Province, China) in September 2021. Specifically, 5 samples were collected from different workshops of Kweichow MT Liquor Co., Ltd. (raw materials: sorghum and corn); 5 samples were collected from different workshops of Guizhou LP Liquor Co., Ltd. (raw materials: corn); 5 samples were collected from different workshops of Guizhou YB Liquor Co., Ltd. (raw materials: sorghum). All samples were dried at 50 °C to a constant weight, crushed through a 60 mesh sieve, and stored in a dry environment. Three representative experimental samples (MT, LP, YB) were obtained by mixing equal amounts (1 kg) of the powder from different workshops of the same enterprise.

Folin-Ciocalteau reagent was purchased from Solarbio Science & Technology Co. Ltd. (Beijing, China). Chromatographic grade formic acid and acetonitrile were purchased from Fisher Scientific (Pittsburgh, USA). Polyphenol standards (purity ≥ 98.0 %, HPLC) and pepsin (P7000, powder, ≥250 U/mg solid, sourced from pig stomach) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Yeast paste, peptone, pancreatin (S10031, 1:4000 BR specifications, trypsin activity: ≥4000 U/g, pancreatic amylase activity: ≥7000 U/g, pancreatic lipase activity: ≥4000 U/g), and bile salt (S30895, bile acid content ≥ 60 %, pig) were purchased from Yuanye Biotech Co., Ltd. (Shanghai, China). Tween-80, hematin chloride, vitamin K1, L-cysteine, resazurin sodium salt, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 4,4′-di (1H-1,2,4-triazol-1-yl)-1,1′-biphenyl (TPTZ) and water-soluble vitamin E (Trolox) were purchased from Beijing Solar Science & Technology Co., Ltd (Beijing, China). Acetic acid (A801296, purity ≥ 99.8 %), propionic acid (P816182, purity ≥ 99.5 %), isobutyric acid (I811668, purity ≥ 99.5 %), butyric acid (B802730, purity ≥ 99.5 %), isovaleric acid (I811830, purity ≥ 99.5 %), valeric acid (V820439, purity ≥ 99.5 %), and 2-ethylbutyric acid (E808830, purity ≥ 99.5 %) were purchased from Shanghai Maclean Biochemical Technology Co., Ltd. (Shanghai, China). Stool DNA kit (D4015-01, USA) were purchased from Omega Bio-Tek, Inc. SYBR Premix Ex Taq TM II was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Analytial reagent NaCl, K_2HPO_4, KH_2PO_4, $NaHCO_3$, $MgSO_4{\cdot}7H_2O$ and $CaCl_2{\cdot}6H_2O$ was purchased from Sinopharm Chemical ReagentCo., Ltd. (Beijing, China).

Determination of the base ingredients of distiller's grains

Moisture, protein, dietary fiber, starch, fat, and ash were measured according to Chinese standards (GB/T 5009.3–2016, GB/T 5009.5–2003, GB/T 6434–2006, T/CBJ 004–2018, GB/T 5009.6–2016, GB/T 5009.4–2016).

Extraction and identification of polyphenols from distiller grains

Polyphenols were extracted according to previous studies with some modifications (Wang et al., 2019; Chan et al., 2023). The sample powder (5 g) was added to the flask and immersed in 100 mL of 60 % ethanol. Afterward, the mixture was ultrasonicated at 420 W for 30 min. After extraction, liquid extracts were separated from solids by centrifugation (5,000 g, 4 °C, 10 min). The extraction process was conducted twice. After the extraction of free polyphenols, 200 mL NaOH (2 mol/L) was added to the residue. The mixture system was filled with nitrogen to isolate the oxygen mixture. Subsequently, it was digested in a shaker (180 r/min, 25 °C) for 4 h. After digestion, the pH of the mixed system was adjusted to 2 ± 0.2, and the extraction was repeated by adding ethyl acetate until the supernatant was colorless. The supernatant was collected and stored at −20 °C. Total polyphenols were obtained by summing free and bound polyphenols. The content of polyphenols in the extracts was determined by the Folin-Ciocalteu method. A standard curve was made using gallic acid as the standard sample, and the regression equation was $y = 0.0852\ x\ + 0.0964$, $R^2 = 0.9999$.

The liquid chromatography analysis was performed on a Vanquish UHPLC System (Thermo Fisher Scientific, USA). Chromatography was carried out with an ACQUITY UPLC ® HSS T3 (150 × 2.1 mm, 1.8 μm) (Waters, Milford, MA, USA). The column was maintained at 40 °C. The flow rate and injection volume were set at 0.25 mL/min and 2 μL, respectively. The mobile phases consisted of (B) 0.1 % formic acid in acetonitrile (v/v) and (A) 0.1 % formic acid in water (v/v). Separation was conducted under the following gradient: 0–1 min, 2 % B; 1–9 min, 2 % − 50 % B; 9–12 min, 50 % − 98 % B; 12–13.5 min, 98 % B; 13.5–14 min, 98 % − 2 % B; 14–20 min, 2 % B. Mass spectrometric detection of metabolites was performed on a Q Exactive (Thermo Fisher Scientific, USA) with an ESI ion source. Simultaneous MS1 and MS/MS (full MS-ddMS2 mode, data-dependent MS/MS) acquisition was used. The parameters were as follows: sheath gas pressure, 30 arb; aux gas flow, 10 arb; spray voltage, 3.50 kV and −2.50 kV for ESI (+) and ESI (-), respectively; capillary temperature, 325 °C; MS1 range, *m/z* 100–1000; MS1 resolving power, 70,000 FWHM; number of data dependent scans per cycle, 10; MS/MS resolving power, 17,500 FWHM; normalized collision energy, 30 eV; dynamic exclusion time, automatic.

Antioxidant activity assay

The antioxidant activity was measured according to a previous reference with some modifications (Huang et al., 2022). Determination of DPPH: The polyphenol extract (1 mL) was mixed thoroughly with methanol solution (6 mL) and centrifuged at 4,000 × *g* for 10 min.

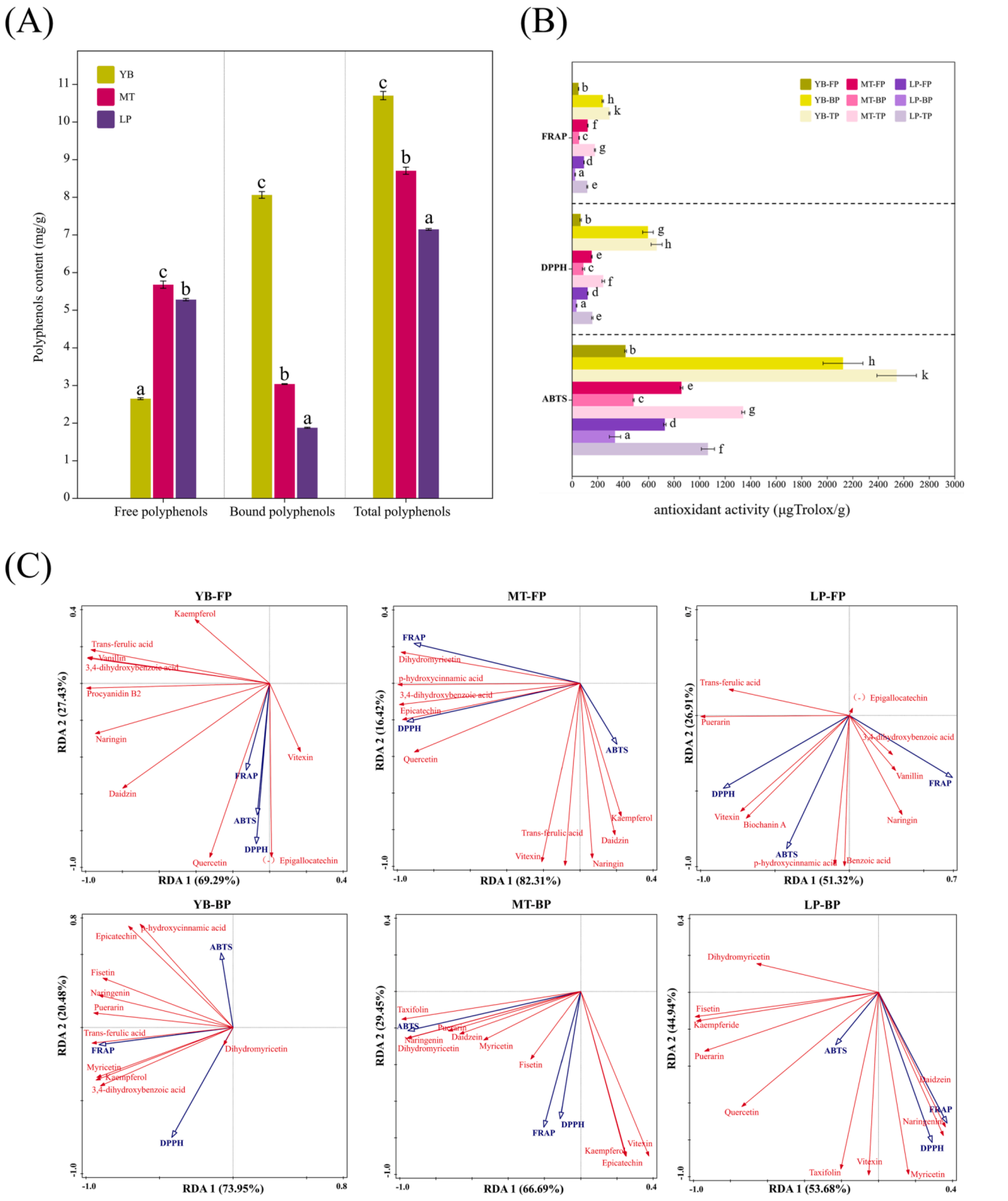

Fig. 1. Analysis of polyphenols content and antioxidant activity of three distiller's grains (YB, MT, LP). (A) Free, bound and total polyphenols content of three distiller's grains. (B) Antioxidant activity of total, free and bound polyphenols of three distiller's grains. (C) RDA analysis between antioxidant and polyphenols compounds. Different letters indicated significant differences ($p < 0.05$). The polyphenols content was expressed in gallic acid equivalents. Antioxidant activity was expressed in water-soluble vitamin E (Trolox) equivalents. The raw material of YB was sorghum. The raw material of MT was sorghum and corn. The raw material of LP was corn. The angles between the arrows represent positive and negative correlations (acute: positive correlation; obtuse: negative correlation; right angle: no correlation). The length of the arrow line indicates the degree of correlation; the longer the line, the greater the correlation and vice versa. FP, Free polyphenols; BP, Bound polyphenols; TP, Total polyphenols.

Afterward, 100 μL of supernatant was mixed with 1900 μL of DPPH standard solution. The mixed system was protected from light for 30 min, and then the absorbance value was measured at 517 nm. Determination of FRAP: Similar to DPPH, polyphenol extract (1 mL) was mixed thoroughly with distilled water (3 mL). After centrifugation, 50 μL of the supernatant was mixed with 2.45 mL of TPTZ standard solution, and the absorbance value was then measured at 593 nm. Determination of ABTS: Similarly, polyphenol extract (1 mL) was mixed thoroughly with distilled water (6 mL). The supernatant (100 μL) was mixed with ABTS standard solution (2.45 mL). The absorbance value was measured at 734 nm. The standard curve was made using Trolox as the standard, and the regression equations for DPPH, ABTS, and FRAP were obtained as follows: $y = 21.385\ x + 10.912$, $R^2 = 0.9992$; $y = 14.737\ x + 12.426$, $R^2 = 0.9999$; $y = 8.6004\ x - 0.855$, $R^2 = 0.9997$.

In vitro digestion fermentation process

The *in vitro* assay was carried out according to the method of a previous study (Cheng et al., 2020). The pH of 10 mL polyphenol extract (1 mg/mL) was adjusted to 2.0 by adding 280 μL of gastric solution (preparing with 72 mg/mL pepsin, enzyme activity ≥ 250 units/mg solid). Subsequently, the mixture systems were incubated for 1 h in a shaker (180 rpm) at 37 °C. After gastric digestion, the pH was immediately adjusted to 6.5 and further adjusted to 7.4, and 6 mL small intestinal digestion (2 mg/mL bile powder: 10 mg/mL pancreatin = 1: 3) was added to the mixture and incubated for 2 h in a shaker (180 rpm) at 37 °C.

Fresh feces of simulated colonic fermentation came from two healthy donors (BMI: 22.36–24.12) without disease, antibiotic treatment, and prebiotic treatment for at least 3 months before the study. The fresh feces were mixed with sterile phosphate buffer (0.01 mol/L) at 1:9 (w/v) and filtered through gauze to make a fecal suspension. Low-dosage (0.66 mL), medium-dosage (1.32 mL), and high-dosage (2.64 mL) distiller grain polyphenol samples were set during colonic fermentation (Tang et al., 2021). The sterile anaerobic medium was adjusted to pH = 7 and added to the samples at 13.08 mL, 12.66 mL, and 11.82 mL for each dosage. The mixture system was incubated for 48 h at 37 °C under anaerobic conditions (90 % N_2, 5 % CO_2, 5 % H_2). At the end of colonic fermentation, the hybrid system was centrifuged (1,0000 × *g*, 4 °C, 10 min) to obtain precipitates and supernatants, respectively, and stored at −20 °C for DNA extraction and SCFAs analysis.

Fecal microbial DNA extraction and qPCR analysis

Fecal microbial DNA was extracted using the Omega fecal DNA kit. According to a previous study (Tang et al., 2021), specific primers (Supplementary Table S1) for total bacteria, *Escherichia coli*, *Enterococcus*, *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria were used to quantify the quantity of fecal microbiota. The cycling program was as follows: an initial denaturation step at 95 °C for 30 s, denaturation at 95 °C for 5 s, annealing at 53 °C for 30 s, and extension at 72 °C for 30 s. Afterward, fluorescence was collected during the extension phase, and the melting curve was measured when the temperature increased from 60 °C to 95 °C. The relative expression of the fecal microbiota was analyzed using the $2^{-\Delta\Delta Ct}$ method, ΔCt = Ct (target gene) - Ct (housekeeping gene); ΔΔCt = ΔCt (experimental treatment) - ΔCt (control treatment).

The SCFAs analysis

The colonic fermented supernatant (0.8 mL) was mixed with 0.16 mL of H_2SO_4 (50 %, v/v) and 80 μL of 2-ethyl butyric acid (790 μmol/L) for 10 min, and the mixture systems were acidified (4 °C, 1 h). Afterward, 0.8 mL ethyl acetate was added and vortexed for 5 min. The mixture systems were incubated at 4 °C for 10 min and centrifuged at 15,000 × *g* for 5 min. The chromatographic column type was DB-WAX (30 m × 0.25 mm × 0.25 μm). The flame ionization detector was set at 250 °C, the flow rate at 1 mL/min, the injection volume at 1 μL, and the splitting ratio at 10: 1. The analysis procedure for SCFAs was as follows: initial temperature of 105 °C for 3 min, heating to 170 °C at a rate of 10 °C/min, followed by heating to 240 °C at a rate of 10 °C/min for 7 min.

Statistical analysis

All data were statistically analyzed using IBM SPSS Statistics 26, and the results are expressed as the mean ± standard deviation. Significant differences among the samples were determined by one-way ANOVA (Duncan's) and Pearson correlation analysis, with significance at $p < 0.05$.

Results and discussion

Polyphenol content in the distiller's grains

The sample's total, free, and bound polyphenol contents are shown in Fig. 1A. Polyphenol content showed significant differences ($p < 0.05$) among different samples. In this study, the total polyphenol content (7.14 mg/g − 10.78 mg/g) was higher than the reported total polyphenol content (3.50 mg/g − 6.80 mg/g) in previous studies (Wang et al., 2019). Polyphenols of distiller grains were mainly derived from brewing grains, and the content of polyphenols varied considerably from one brewing grain to another (Hung, 2016), YB (10.78 ± 0.11 mg/g), MT (8.70 ± 0.09 mg/g), and LP (7.14 ± 0.03 mg/g). In addition, the extraction method affected the polyphenol content. Ultrasound-assisted combined with alkaline digestion obtained higher of polyphenols: sonication resulted in a 14.29 % increase in distiller's grain polyphenol extraction, and alkaline digestion promoted the release of polyphenols in the bound state (Izadifar, 2013).

Bound and free polyphenol contents showed significant differences ($p < 0.05$) among different samples. The crushing of the brewing grains promoted the conversion of bound polyphenols to free polyphenols (Sharma et al., 2012). During brewing, YB preserved intact sorghum grains, while MT and LP were crushed into powder, which resulted in higher levels of bound polyphenols in YB (8.05 ± 0.09 mg/g) than in MT (3.03 ± 0.01 mg/g) and LP (1.88 ± 0.02 mg/g), while MT (5.67 ± 0.10 mg/g) and LP (5.27 ± 0.04 mg/g) had higher levels of free polyphenols than YB (2.47 ± 0.02 mg/g). The starch and dietary fiber contents of YB were higher than those of MT and LP, at 22.73 ± 0.57 g/100 g and 16.22 ± 0.95 g/100 g (Supplementary Table S2), respectively, which might also account for the high bound polyphenol content of YB. Distiller's grains high in starch and dietary fiber might have more bound polyphenols, attributing to that polyphenols were always bound to dietary fiber or starch in strong covalent bonds to form complexes (Zhang et al., 2023). Furthermore, distiller's grains are rich in nutrients such as starch, protein, and dietary fiber (Supplementary Table S2), broadening their potential applications. By utilizing the starch content, distiller's grains could be used to produce liquor with a refreshing body and harmonized aroma by adding enzymes, active yeasts, and *Daqu* (Liu et al., 2022). Employing microorganisms with high-efficiency enzyme production, non-toxic properties, high protein content in cells, and rapid reproduction speed to ferment distiller's grains can yield protein feed (Wang et al., 2015; Liu et al., 2022). Moreover, distiller's grains are rich in dietary fiber, which has the function of reducing the risk of diabetes and enhancing intestinal absorption (Liu et al., 2022). The above analysis showed that YB had higher levels of bound polyphenols than MT and LP, while MT and LP had more free polyphenols. The crushing of brewing grains facilitated the conversion of bound polyphenols into free polyphenols.

Table 1
Composition and concentration of the distiller's grains polyphenols.

Name	R_t (min)	Formula	M/Z	Exact mass	Error (ppm)	Precursor type	Concentration(μg/g) YB-FP	YB-BP	MT-FP	MT-BP	LP-FP	LP-BP
Trans-ferulic acid	6.01	$C_{10}H_{10}O_4$	193.0474	194.0579	1.773	$[M-H]^-$	6.24 ± 0.20^a	13.27 ± 1.40^b	39.14 ± 0.03^c	3.23 ± 0.41^a	64.88 ± 11.97^d	2.43 ± 0.37^a
4-hydroxybenzoic acid	11.21	$C_7H_5O_3$	137.0239	138.0317	3.649	$[M-H]^-$	2.28 ± 0.22^b	2.45 ± 0.71^b	5.18 ± 0.40^c	0.56 ± 0.06^a	7.75 ± 1.12^c	0.43 ± 0.10^a
Hydrocinnamic acid	9.92	$C_9H_8O_2$	149.0596	150.0681	2.212	$[M-H]^-$	ND	ND	0.27 ± 0.14^a	ND	0.20 ± 0.03^a	ND
Trans-cinnamic acid	7.71	$C_9H_8O_2$	149.0603	148.0525	3.354	$[M+H]^+$	3.24 ± 0.03^b	0.48 ± 0.02^a	4.29 ± 0.18^c	0.58 ± 0.03^a	7.51 ± 0.26^d	0.51 ± 0.02^a
Vanillic acid	6.63	$C_8H_8O_4$	169.0498	168.0423	1.183	$[M+H]^+$	1.08 ± 0.09^b	0.11 ± 0.00^a	3.11 ± 0.20^c	0.92 ± 0.02^b	5.56 ± 0.38^d	1.25 ± 0.05^b
Vanillin	7.62	$C_8H_8O_3$	153.0539	152.0473	3.920	$[M+H]^+$	11.28 ± 0.18^d	0.88 ± 0.03^a	11.97 ± 0.50^d	2.72 ± 0.17^b	24.66 ± 1.21^e	4.60 ± 0.26^c
p-hydroxycinnamic acid	9.76	$C_9H_8O_3$	147.0434	164.0473	2.734	$[M+H-H_2O]^+$	3.53 ± 0.63^a	14.52 ± 0.01^d	147.42 ± 3.00^e	7.59 ± 0.43^c	272.50 ± 23.16^f	5.83 ± 0.18^b
Benzoic acid	3.82	$C_7H_6O_2$	123.0442	122.0368	0.063	$[M+H]^+$	3.26 ± 0.45^{ab}	3.96 ± 0.33^b	3.02 ± 0.12^{ab}	2.86 ± 0.62^a	21.26 ± 0.11^c	3.00 ± 0.11^{ab}
Protocatechualdehyde	6.17	$C_7H_6O_3$	139.0384	138.0317	4.142	$[M+H]^+$	0.82 ± 0.08^b	0.68 ± 0.15^b	9.76 ± 0.36^c	0.61 ± 0.05^b	10.87 ± 0.67^c	0.18 ± 0.01^a
3,4-dihydroxybenzoic acid	2.95	$C_7H_6O_4$	153.0174	154.0266	0.207	$[M-H]^-$	26.94 ± 0.68^d	10.34 ± 1.25^c	26.00 ± 5.71^d	7.21 ± 1.57^b	37.21 ± 8.66^e	5.80 ± 0.88^a
Caffeic acid	5.75	$C_9H_8O_4$	181.0499	180.0423	2.016	$[M+H]^+$	0.69 ± 0.00^a	ND	0.22 ± 0.00^a	ND	0.38 ± 0.03^a	ND
Syringic acid	6.70	$C_9H_{10}O_5$	199.0601	198.0528	0.000	$[M+H]^+$	ND	ND	ND	0.30 ± 0.00^a	ND	0.40 ± 0.01^a
4-hydroxy-3,5-dimethoxycinnamic acid	7.63	$C_{11}H_{12}O_5$	207.0653	224.0685	2.448	$[M+H-H_2O]^+$	0.44 ± 0.01^a	1.09 ± 0.04^b	3.82 ± 1.09^c	0.45 ± 0.01^a	5.79 ± 0.27^d	0.47 ± 0.16^a
Salicylic acid	4.58	$C_7H_6O_3$	137.0227	138.0317	1.966	$[M-H]^-$	0.69 ± 0.05^c	0.10 ± 0.00^a	2.25 ± 0.30^d	0.33 ± 0.06^b	5.20 ± 0.39^e	0.32 ± 0.02^b
Puerarin	8.87	$C_{21}H_{20}O_9$	417.1175	416.1107	1.141	$[M+H]^+$	0.81 ± 0.09^a	15.39 ± 2.63^{bc}	0.79 ± 0.06^a	18.78 ± 1.19^c	12.70 ± 3.21^b	35.75 ± 0.46^d
Epicatechin	6.40	$C_{15}H_{14}O_6$	291.0856	290.0790	2.322	$[M+H]^+$	0.78 ± 0.14^a	212.94 ± 22.54^e	15.84 ± 2.29^c	196.68 ± 8.49^d	5.06 ± 0.57^b	1.34 ± 0.01^a
Dihydromyricetin	5.63	$C_{15}H_{12}O_8$	303.0491	320.0532	1.002	$[M+H-H_2O]^+$	0.19 ± 0.02^a	10.42 ± 0.43^c	12.02 ± 1.38^c	33.36 ± 1.25^d	1.34 ± 0.61^b	62.77 ± 0.66^e
Vitexin	12.67	$C_{21}H_{20}O_{10}$	432.2366	432.1056	0.373	$[M]^+$	9.64 ± 0.61^b	0.35 ± 0.01^a	356.66 ± 10.33^f	24.89 ± 0.86^c	144.29 ± 4.92^e	46.43 ± 1.21^d
Quercetin 3-glucoside	7.16	$C_{21}H_{20}O_{12}$	465.1008	464.0955	3.779	$[M+H]^+$	ND	0.97 ± 0.10^a	2.33 ± 0.23^b	3.10 ± 0.36^b	4.48 ± 0.66^{bc}	5.44 ± 0.26^c
Taxifolin	7.76	$C_{15}H_{12}O_7$	305.0647	304.0583	2.872	$[M+H]^+$	ND	6.56 ± 0.08^b	6.79 ± 0.45^b	32.42 ± 1.97^c	0.20 ± 0.03^a	55.63 ± 2.20^d
Naringin	8.21	$C_{27}H_{32}O_{14}$	563.1549	580.1792	2.949	$[M+H-H_2O]^+$	89.38 ± 2.79^c	5.53 ± 0.32^a	114.65 ± 8.35^d	5.20 ± 1.35^a	255.09 ± 4.68^e	8.02 ± 0.78^b
Myricetin	7.39	$C_{15}H_{10}O_8$	301.0265	318.0376	1.178	$[M+H-H_2O]^+$	0.19 ± 0.01^a	21.26 ± 0.81^d	2.24 ± 0.05^b	11.16 ± 0.12^c	0.71 ± 0.23^a	20.35 ± 0.60^d
Fisetin	5.97	$C_{15}H_{10}O_6$	287.0551	286.0477	0.000	$[M+H]^+$	1.55 ± 0.26^a	280.68 ± 6.88^d	9.99 ± 0.89^b	201.4 ± 10.12^c	4.98 ± 0.72^a	413.55 ± 4.69^e
Daidzein	9.09	$C_{15}H_{10}O_4$	254.0566	254.0579	0.117	$[M]^+$	3.97 ± 0.54^b	4.68 ± 0.22^b	18.21 ± 1.27^c	20.59 ± 1.27^c	0.22 ± 0.04^a	27.42 ± 1.01^d
Quercetin	9.41	$C_{15}H_{10}O_7$	301.0348	302.0427	0.385	$[M-H]^-$	4.11 ± 0.71^a	1.50 ± 0.06^a	538.82 ± 28.54^d	5.34 ± 0.52^a	9.19 ± 1.62^b	38.28 ± 1.32^c
Naringenin	10.12	$C_{15}H_{12}O_5$	273.0749	272.0685	2.930	$[M+H]^+$	1.29 ± 0.12^a	22.90 ± 2.14^b	1.63 ± 0.27^a	137.68 ± 12.47^c	0.68 ± 0.18^a	339.58 ± 2.21^d
Kaempferol	5.27	$C_{15}H_{10}O_6$	287.0551	286.0477	0.299	$[M+H]^+$	33.46 ± 1.14^b	30.89 ± 1.34^b	22.51 ± 1.61^b	355.04 ± 11.7^c	8.26 ± 0.60^a	5.02 ± 0.26^a
Kaempferide	10.32	$C_{16}H_{12}O_6$	301.0697	300.0634	0.108	$[M+H]^+$	0.46 ± 0.03^a	0.66 ± 0.25^a	1.41 ± 0.41^a	8.51 ± 0.53^c	4.89 ± 0.78^b	10.03 ± 0.78^d
Biochanin A	9.99	$C_{16}H_{12}O_5$	285.2057	284.0685	4.962	$[M+H]^+$	3.01 ± 0.08^a	ND	8.15 ± 0.05^b	2.28 ± 0.22^a	16.17 ± 0.17^c	2.69 ± 0.48^a
Pelargonidin chloride	6.60	$C_{15}H_{11}ClO_5$	271.0596	271.0606	3.689	$[M]^+$	ND	0.16 ± 0.02^a	0.13 ± 0.00^a	0.78 ± 0.18^a	ND	4.00 ± 0.19^b
Cyanidin 3-galactoside	12.47	$C_{21}H_{21}ClO_{11}$	449.1478	449.1084	1.067	$[M]^+$	ND	0.25 ± 0.06^a	ND	0.86 ± 0.14^a	ND	1.49 ± 0.30^b
Isorhamnetin	9.31	$C_{16}H_{12}O_7$	317.0657	316.0583	0.315	$[M+H]^+$	ND	0.37 ± 0.03^a	ND	ND	ND	0.28 ± 0.02^a
Procyanidin B2	5.53	$C_{30}H_{26}O_{12}$	579.1489	578.1424	0.159	$[M+H]^+$	19.93 ± 0.18^c	0.15 ± 0.00^a	9.04 ± 0.06^b	ND	6.41 ± 0.88^b	0.25 ± 0.00^a
(-) epigallocatechin	1.76	$C_{15}H_{14}O_7$	305.0662	306.0740	1.563	$[M-H]^-$	94.80 ± 2.24^c	1.97 ± 0.05^a	2.37 ± 0.16^a	10.70 ± 2.20^b	129.57 ± 15.3^d	3.61 ± 0.96^a

The composition and concentration of polyphenols compounds in free polyphenols and bound polyphenols of three distillers' grains (YB, MT, LP). Data were expressed as the mean ± standard. Means with different letters in each row were significantly different at $p < 0.05$. FP, Free polyphenols; BP, Bound polyphenols; Rt, Retention

time; $[M-H]^-$, Precursor ion obtained from mass spectrum in negative mode; $[M+H]^+$, $[M]^+$, and $[M+H-H_2O]^+$, Precursor ion obtained from mass spectrum in positive mode.

Identification of polyphenols in the distiller's grains by UPLC-ESI/QE-MS/MS

The polyphenol compounds in the distiller's grains were characterized and quantified using UPLC-ESI/QE-MS/MS (Table 1). A total of 165 polyphenol compounds were identified in the free and bound polyphenols of the three distiller grains, and 34 of these compounds were quantified. Quercetin, syringic acid, vanillic acid, caffeic acid, *p*-hydroxycinnamic acid, epicatechin, and 4-hydroxy-3,5-dimethoxycinnamic acid were detected in distiller grains, which was consistent with previous study (Wang et al., 2019). Cereal was rich in ferulic acid, while ferulic acid was not detected in all samples, which was probably due to the altered polyphenol composition and concentration of the brewing grains after crushing, fermentation, and distillation (Carvalho & Guido, 2022). Microorganisms produce hydrolytic enzymes such as xylanase, cellulase, and β-glucosidase to effectively degrade insoluble dietary fiber structure and release polyphenol compounds covalently bound to insoluble dietary fiber (Liao et al., 2022). Additionally, it converts polyphenols via the hydroxycinnamic acid pathway, phenyl acid pathway, and benzoic acid pathway (Liao et al., 2022). For example, gallic acid, protocatechuic acid, caffeic acid, and ferulic acid were detected by-products fermented by *Lactobacillus casei* as compared to unfermented pomace, and it is hypothesized that it may be produced by metabolizing anthocyanins by *Lactobacillus casei* (Cheng et al., 2020). After fermentation of black tartary buckwheat, the contents of quercetin and epicatechin increased approximately 13.67-fold and 8.51-fold, respectively. Notably, kaempferol and chlorogenic acid, which were not detected in the raw material, were detected after fermentation and were as high as 285.19 ± 3.03 mg/kg and 36.71 ± 0.63 mg/kg, respectively (Ren et al., 2021). Phenolic acids (vanillic acid, salicylic acid, syringic acid) and anthocyanins (pelargonidin chloride, cyanidin 3-galactoside, isorhamnetin, procyanidin B2, etc.) were detected in the free and bound polyphenols of all samples. Similarly, quercetin, 3,4-dihydroxybenzoic acid, epicatechin, myricetin, and isorhamnetin were identified in the free and bound polyphenols of three distiller grain samples. The content of quercetin in the free polyphenols of MT was highest among all samples, up to 538.82 ± 28.54 μg/g. Quercetin had vigorous antioxidant activity due to its unique molecular structure (Boots et al., 2008), which implied that quercetin played an important role in the antioxidant activity of distiller's grain polyphenols. In addition, the content of epicatechin in the bound polyphenols of BP was highest in all samples, up to 212.94 ± 22.54 μg/g. In previous study, epicatechin has been reported to have a strong antioxidant capacity (Qu et al., 2021). Altogether, distillers' grains contain abundant phenolic compounds, which lays the foundation for realizing the high-value application and good active functions of distillers' grains.

Antioxidant activity and correlation analysis

The antioxidant activity of distiller's grain polyphenols was shown in Fig. 1B. The antioxidant activity of different distiller grains showed significant differences ($p < 0.05$). The FRAP (120.38 ± 4.07 μg Trolox/g − 294.02 ± 4.47 μg Trolox/g), DPPH (159.89 ± 5.65 μg Trolox/g − 664.54 ± 42.54 μg Trolox/g), and ABTS (1066.74 ± 50.60 μg Trolox/g − 2546.28 ± 152.06 μg Trolox/g) of the distiller's grains were within the range of those already reported in a previous study (Shin et al., 2018). The antioxidant activity of total and bound polyphenols was higher in YB than in the other samples. Previous studies have shown that sorghum polyphenols had higher antioxidant activity than other grains (wheat, corn, rice) (Gong et al., 2018). The free polyphenols of MT had higher antioxidant activity than the other samples, which might be closely related to its polyphenol composition. The UPLC-ESI/QE-MS/MS results (Table 1) showed that free polyphenols of MT had the highest quercetin content (538.82 ± 28.54 μg/g) among all samples. Quercetin has vigorous antioxidant activity due to its unique molecular structure (catechol group in the B ring; 2,3-double bond conjugated with a 4-oxo group in the C ring; hydroxyl group at C3 and C5 positions in the heterocyclic ring) (Boots et al., 2008). Quercetin could exert antioxidant activity by inhibiting the production of reactive oxygen species, chelating metal ions, increasing the activity of antioxidant enzymes, and activating the mitogen-activated protein kinase pathway (Xu et al., 2019). In comparation with catechins and rutin, quercetin scavenged more free radicals and chelated metal ions, exhibiting a measurement dependency effect (Hung, 2016). This might be the reason for the high antioxidant activity of the free polyphenols of MT.

Moreover, several studies have reported a positive correlation between antioxidant activity and polyphenol content (Tang et al., 2021; Cheng et al., 2020). The consistent trend between polyphenol content and antioxidant activity in Fig. 1 suggests a strong relationship between the two variables. This implies that the antioxidant activity of a sample might be largely influenced by its polyphenol content. Furthermore, the content of epicatechin and myricetin in bound polyphenols of YB was significantly higher than those in other samples. The content of epicatechin was 1.08–273.00 times higher than that in other samples, while the content of myricetin was 1.99–111.89 times higher than that in other samples (Table 1). Previous studies have indicated that catechins and myricetin exhibit significant antioxidant activity. For example, myricetin and epicatechin can effectively protect against the oxidative rancidity of food, and the antioxidant properties are superior to those of the common synthetic antioxidant and caffeic acid (Ruan et al., 2022). They can prevent oxidative stress and protect against cellular oxidative damage by increasing the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase, and hydrogen peroxide (Hassana et al., 2017). Therefore, the antioxidant activity may be related to the content and composition of polyphenols.

Redundancy analysis (Fig. 1C) showed a positive correlation between quercetin and antioxidant activity in free polyphenols of MT, free polyphenols of YB, and bound polyphenols of LP, consistent with a previous study (Baldermann et al., 2018). In the bound polyphenols of YB, MT and LP, myricetin and naringenin were positively correlated with antioxidant activity. Furthermore, in the free polyphenols of MT and the bound polyphenols of YB with strong antioxidant activity, epicatechin and myricetin showed better positive correlations with antioxidant activity. This meant that these polyphenol compounds were one of the main contributors to the antioxidant activity. Previous study have indicated a significant positive correlation between epicatechin, epicatechin gallate, epigallocatechin gallate, and antioxidant activity, which are considered the primary antioxidants (Ma et al., 2022). Epicatechin has a positive effect on antioxidant activity, which not only reduces oxidative damage by modulating key control factors involved in oxidative stress and increasing the activity of superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase but also reduces reactive oxygen species production by modulating the phosphatidylinositol 3-kinase/protein kinase B pathway to reduce reactive oxygen species production (Qu et al., 2021). Qi et al. also showed that myricetin is a potent antioxidant that reduces reactive oxygen species production by inhibiting the JAK/STAT1 and NOX2/p47phox pathways to protect against acute lung injury in mice (Qi et al., 2017). The results showed that free polyphenols of MT and bound polyphenols of YB had high antioxidant activity, and the antioxidant activity was consistent with the trend of the polyphenol content. The level of antioxidant activity was related to the brewing grain and polyphenol composition. Quercetin, myricetin, epicatechin, and naringenin might have an important contribution to antioxidant activity.

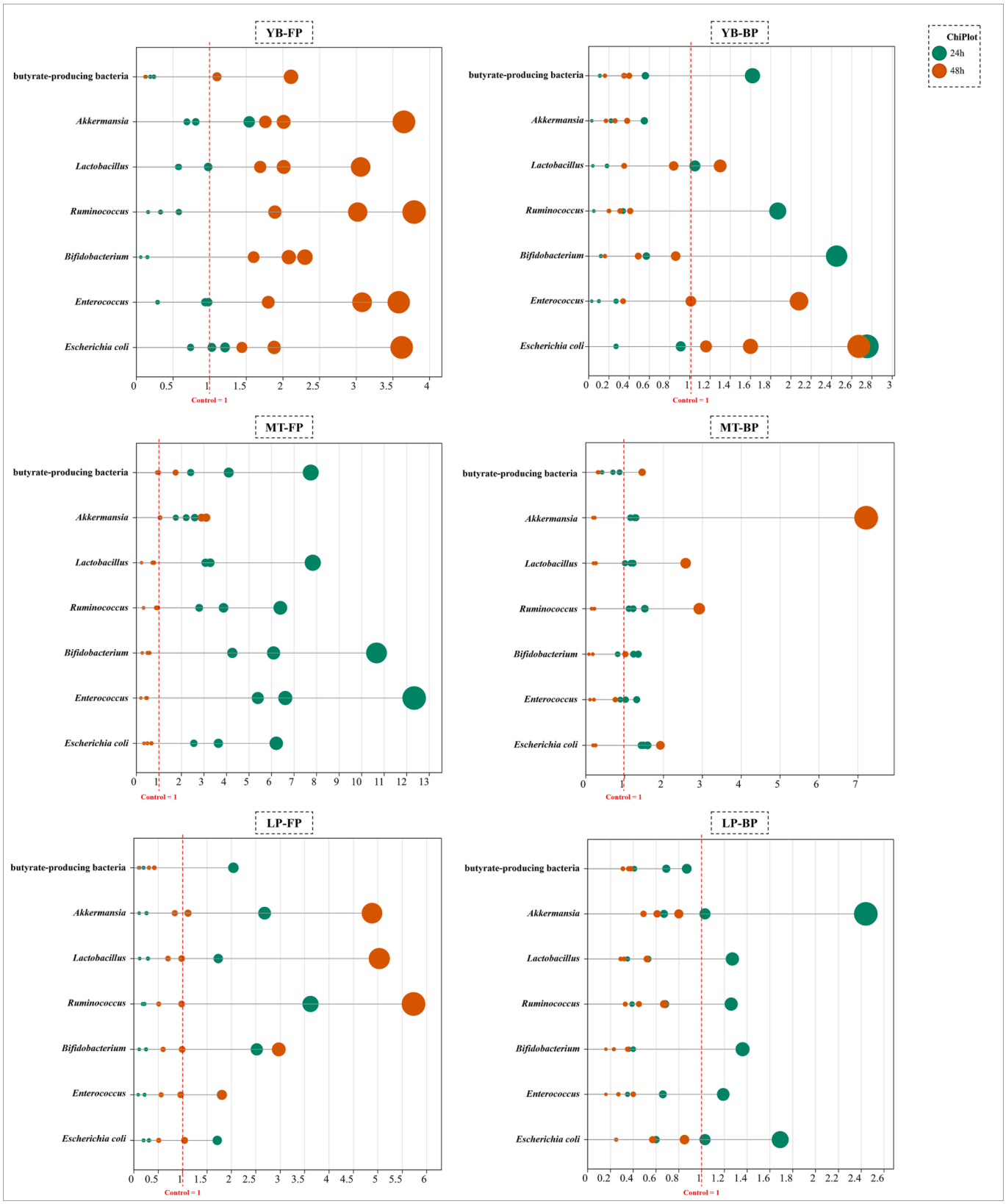

Fig. 2. Influence of free and bound polyphenols from distiller's grains (YB, MT, LP) on the gut microbiota during the simulated colonic fermentation 24 h and 48 h. A control group, a low-dose group, a medium-dose group, and a high-dose group were set up respectively. ">1" represented promotion, and "<1" represented inhibition. FP, Free polyphenols; BP, Bound polyphenols.

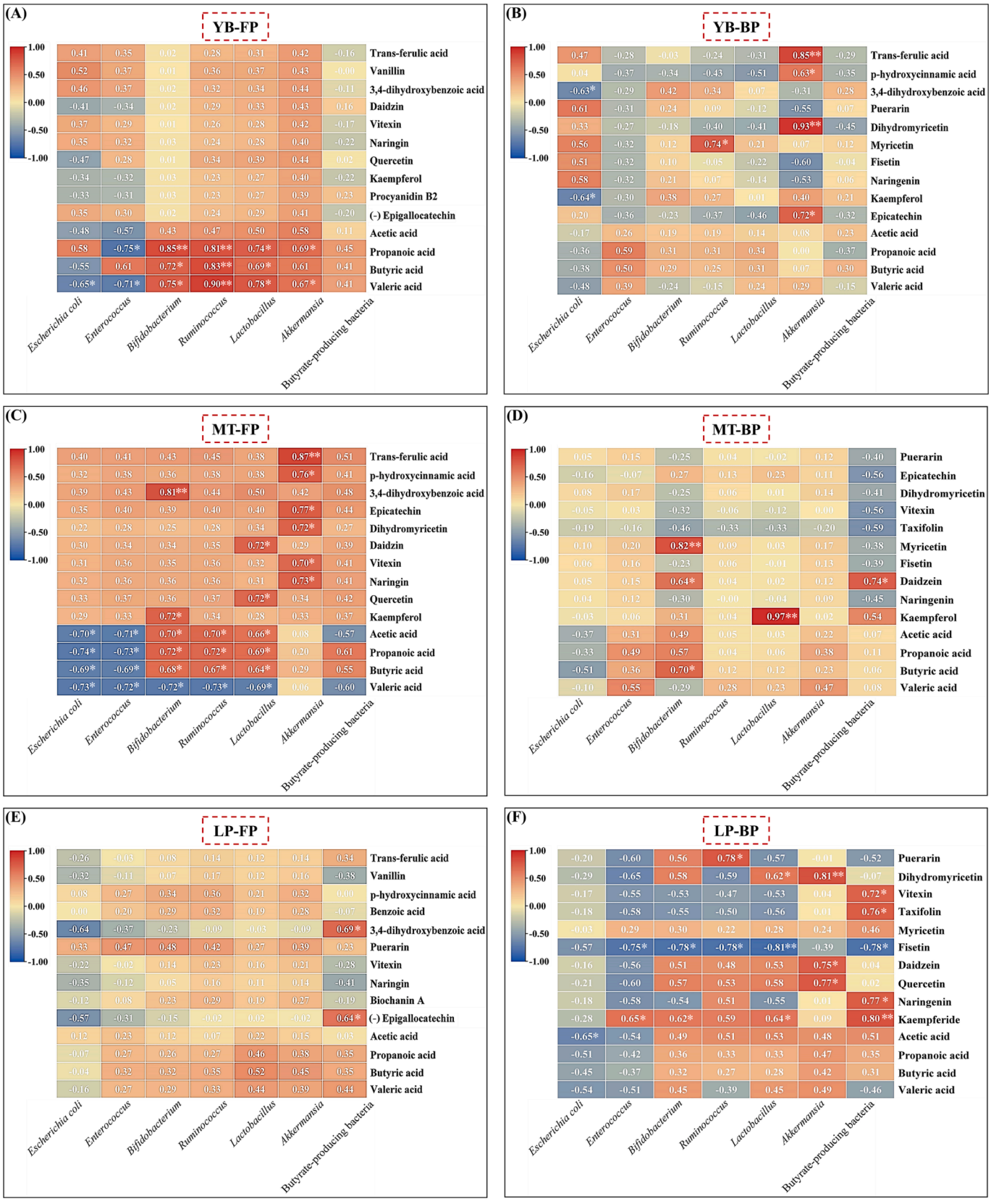

Fig. 3. Association analysis of gut microbiota with SCFAs and main polyphenols compounds. ">0" represented positive correlation, and "<0" represented negative correlation. * Correlation was significant at $p < 0.05$. ** Correlation was extremely significant at $p < 0.01$. FP, Free polyphenols; BP, Bound polyphenols.

Changes in fecal microbiota by distiller's grain polyphenols

The results of A260/A280, A260/A230, and agarose gel electrophoresis are shown in Supplementary Fig. S1. The ratios of A260/A280 and A260/A230 ranged from 1.8 to 2.2 and 1.8 to 2.3, indicating good purity and integrity of the DNA. The effect of the distiller's grain polyphenol treatment on the relative abundance of fecal microorganisms is shown in Fig. 2. The distiller's grain polyphenol treatments generally reduced ($p < 0.05$) the relative abundance of *Escherichia coli* and *Enterococcus*. Free and bound polyphenols from MT and LP samples significantly reduced ($p < 0.05$) the relative abundance of *Escherichia coli* and *Enterococcus* at 48 h of simulated fermentation. Similarly, free and bound polyphenols of YB inhibited ($p < 0.05$) the abundance of *Escherichia coli* and *Enterococcus* at 24 h of simulated fermentation. Since *Escherichia coli* and *Enterococcus* are associated with intestinal endotoxins and the acquisition of virulence factors, excessive development of *Escherichia coli* and *Enterococcus* is considered harmful to intestinal health (Tang et al., 2021; Cheng et al., 2020). Decreased abundance of *Escherichia coli* and *Enterococcus* might be associated with polyphenol composition. The free and bound polyphenols of the distiller's grains were rich in quercetin, chlorogenic acid, and anthocyanins (Table 1). In previous studies, quercetin, chlorogenic acid, and anthocyanins inhibited the growth of potentially pathogenic bacteria such as *Escherichia coli* and *Enterococcus* (Chan et al., 2023); polyphenols inhibited the growth of *Escherichia coli* and *Staphylococcus* by disrupting the lipid bilayer of cell membranes or altering the permeability of cell membranes (Xie et al., 2015). Moreover, the gut microecology is a dynamically balanced environment where changes in the number of intestinal probiotics govern the number of conditionally pathogenic microbiota (Cheng et al., 2020; Li et al., 2023). The growth of intestinal probiotics might hinder the growth and multiplication of *Escherichia coli* and *Enterococcus*. The intestinal probiotics not only consume a large amount of resources, resulting in a lack of nutrients for pathogenic bacteria to grow, but they also secrete anti-microbial substances, such as hydrogen peroxide, bacteriocins, and organic acids, which directly inhibit or destroy pathogenic bacteria (Rawal & Ali, 2023).

Free polyphenols of MT and LP rose ($p < 0.05$) the relative abundance of *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria at 24 h of simulated fermentation. In comparation with the control group, free polyphenols of MT significantly rose ($p < 0.05$) the relative abundance of *Bifidobacterium*, *Ruminobacterium*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria by approximately 10.66 ± 0.83-, 6.39 ± 0.05-, 7.83 ± 0.09-, 2.59 ± 0.83-, and 7.74 ± 0.76-fold, respectively (Supplementary Table S3). Similarly, bound polyphenols of YB, MT and LP and free polyphenols of YB rose ($p < 0.05$) the relative abundance of these beneficial bacteria. In this study, anthocyanins, quercetin, and chlorogenic acid were identified in all samples (Table 1). Anthocyanins, quercetin, and chlorogenic acid have been shown to increase the abundance of intestinal probiotics and inhibit the abundance of potential pathogenic bacteria (Xie et al., 2022). Moreover, the bioavailability was one of the factors influencing the impact on the gut microbiota, and polyphenol compounds with glycosides in their structure generally had a high bioavailability (Cheng et al., 2020). Direct oral administration of quercetin and its O-glycoside derivatives to rats showed that the bioavailability of isoquercitrin and quercetin was higher than that of rutin (Makino et al., 2013). This might imply that polyphenol compounds with high bioavailability had a greater effect on gut microbiota than other compounds. Moreover, distiller's grains are rich in other active substances such as dietary fiber and polyunsaturated fatty acids (Liu et al., 2022). They could regulate the gut microbiota by stimulating the growth of beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, *Akkermansia*, and butyrate-producing bacteria and hindering pathogenic bacteria (Tang et al., 2021; Zapata et al., 2022).

Free polyphenols of MT showed the most significant promotion effect on *Lactobacillus* (7.83 ± 0.09-fold) and *Bifidobacterium* (10.66 ± 0.83-fold) in all samples. The improvement of *Lactobacillus* and *Bifidobacterium* by free polyphenols of MT might be related to more abundant macromolecular polyphenols (structures containing glycosidic ligands and polymers) than other samples, such as quercetin, hesperetin, naringin, naringenin, neohesperidin, daidzein, and diosmin. In a previous study, it was noted that large-molecule polyphenols were the main factor affecting the abundance of gut microbiota (Tang et al., 2021; Cheng et al., 2020). Macromolecule polyphenols were metabolized by various enzymes produced by gut microbiota as substrates in the catalytic reactions to produce corresponding aglycones, thereby promoting the growth of the corresponding bacteria. For example, quercetin, naringin, naringenin, hesperetin, isoquercitrin, hesperidin, hesperetin-7-O-glucoside, and prunin changed the structure of gut microbiota by increasing the relative abundance of intestinal probiotics (*Bifidobacterium*, *Lactobacillus*), and decreasing the relative abundance of potential pathogenic bacteria (*Lachnoclostridium*, *Bilophila*) (Pan et al., 2023); oral administration of polyphenol solutions to mice significantly increased the abundance of *Lactobacillus* and *Bifidobacterium* in the contents of the cecum, and *Lactobacillus* and *Bifidobacterium* were shown to have β-glucosidase activity, so they could perform hydrolysis reactions on polyphenols (Chen et al., 2021). Taken together, the distiller's grain polyphenol treatment, especially free polyphenols of MT, stimulated intestinal probiotics such as *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermansia*, butyrate-producing bacteria and hindered potentially pathogenic bacteria (*Escherichia coli*, *Enterococcus*).

Correlation analysis was performed to further investigate the interactions between gut microbiota, SCFAs, and polyphenol compounds (Fig. 3). In free polyphenols of MT, SCFAs (acetic acid, propionic acid, butyric acid) were negatively ($p < 0.05$) correlated with *Escherichia coli* and *Enterococcus*, and positively ($p < 0.05$) associated with *Bifidobacterium*, *Ruminococcus*, and *Lactobacillus*. Similarly, in free polyphenols of YB, SCFAs (propanoic acid, butyric acid, valeric acid) showed a significant ($p < 0.05$) positive correlation with *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, and *Akkermansia*, additionally, valeric acid showed significant ($p < 0.05$) negative correlations with *Escherichia coli* and *Enterococcus*. SCFAs are mainly produced by gut microbiota fermenting indigestible carbohydrates in the gut. Meanwhile, SCFAs could promote the growth of the corresponding gut microbiota by providing energy for microbiota metabolism, acidifying the intestinal environment, and maintaining the intestinal environment. For example, *Lactobacilli*, *Bifidobacterium*, *Enterococcus*, and *Bacteroides/Prevotella* were metabolized to produce butyric acid, lowering the pH in the gut, preventing the accumulation of lactic acid, inhibiting the growth of *Clostridium histolyticum* or pathogens sensitive to acidic conditions and promoting the development of butyrate-producing bacteria (Gong et al., 2018; Cheng et al., 2020); acetic acid, propionic acid, and butyric acid were strikingly positively correlated with *Flavonifractor* and *Sutterella* (Pan et al., 2023). In general, SCFAs had an essential role in inhibiting potential pathogenic bacteria and promoting intestinal probiotics.

In addition, quercetin positively correlated with these intestinal probiotics (*Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermansia*, butyrate-producing bacteria) in free polyphenols of MT and YB and bound polyphenols of LP. Quercetin showed a significant ($p < 0.05$) positive correlation with *Lactobacillus* (in free polyphenols of MT) and *Akkermansia* (in free polyphenols of LP). A study showed that black tartary buckwheat probiotic fermentation fermented by *Bacillus* sp. targeted the abundance of *Lactobacillus*, *Faecalibaculum*, and *Allobaculum* by increasing the content of compounds such as quercetin and kaempferol, and there was a significant positive correlation between quercetin and kaempferol and *Allobaculum* (Ren et al., 2021). In bound polyphenols of LP, there was a positive ($p < 0.05$) correlation between polyphenol compounds (dihydromyricetin, daidzein, quercetin) and *Akkermansia*, which was closely related to the increased abundance of *Akkermansia*. Among the free polyphenols of MT, *trans*-ferulic acid, *p*-hydroxycinnamic acid, epicatechin, dihydromyricetin, vitexin, and naringin were significantly positively correlated with *Akkermansia*;

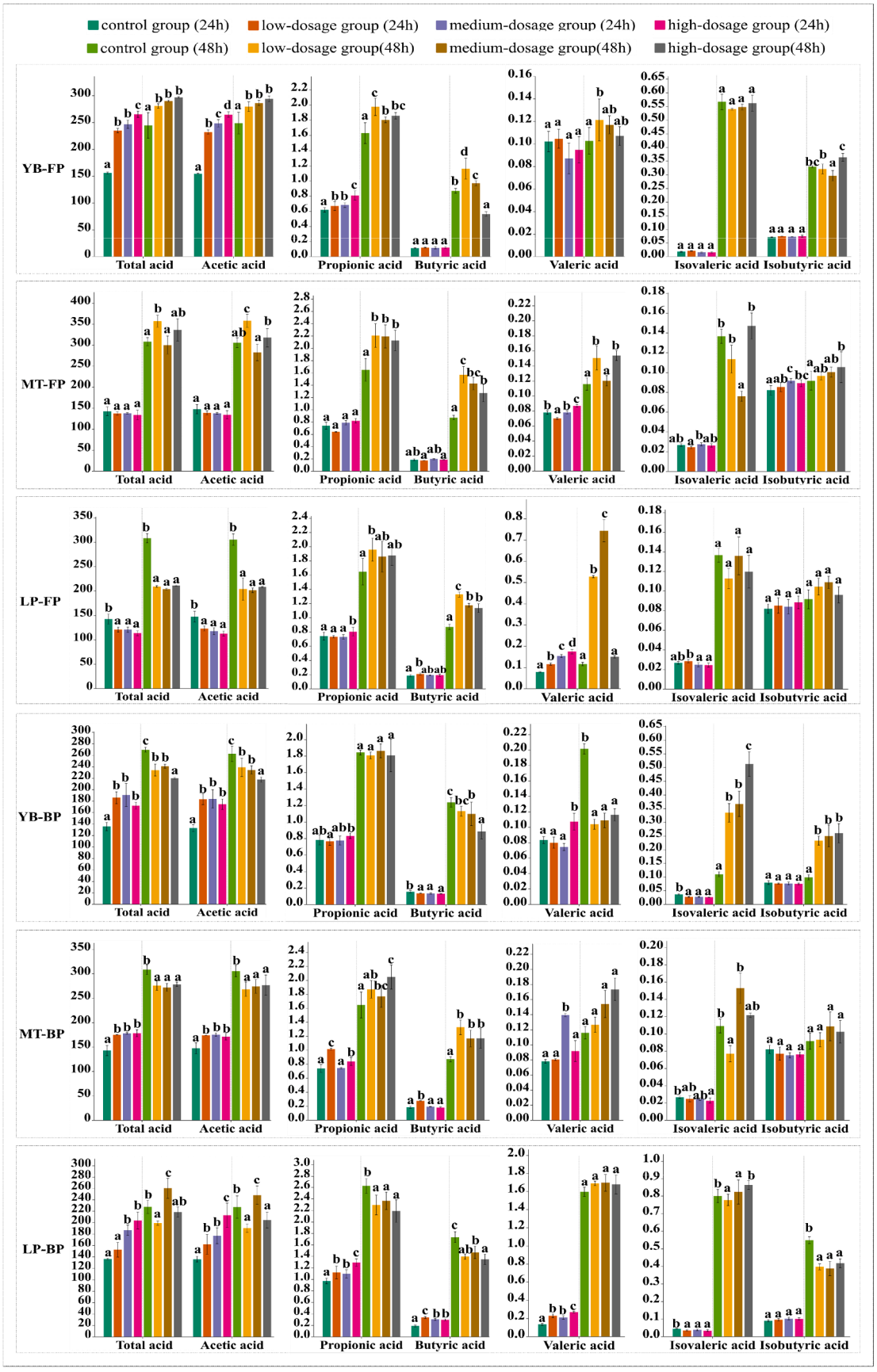

Fig. 4. Influence of free and bound polyphenols from distiller's grains (YB, MT, LP) on the total acid, acetic acid, propanoic acid, butyric acid, valeric acid, isovaleric acid, and isobutyric acid during the simulated colonic fermentation 24 h and 48 h. Different letters indicated significant differences ($p < 0.05$) between control and treatment group. FP, Free polyphenols; BP, Bound polyphenols.

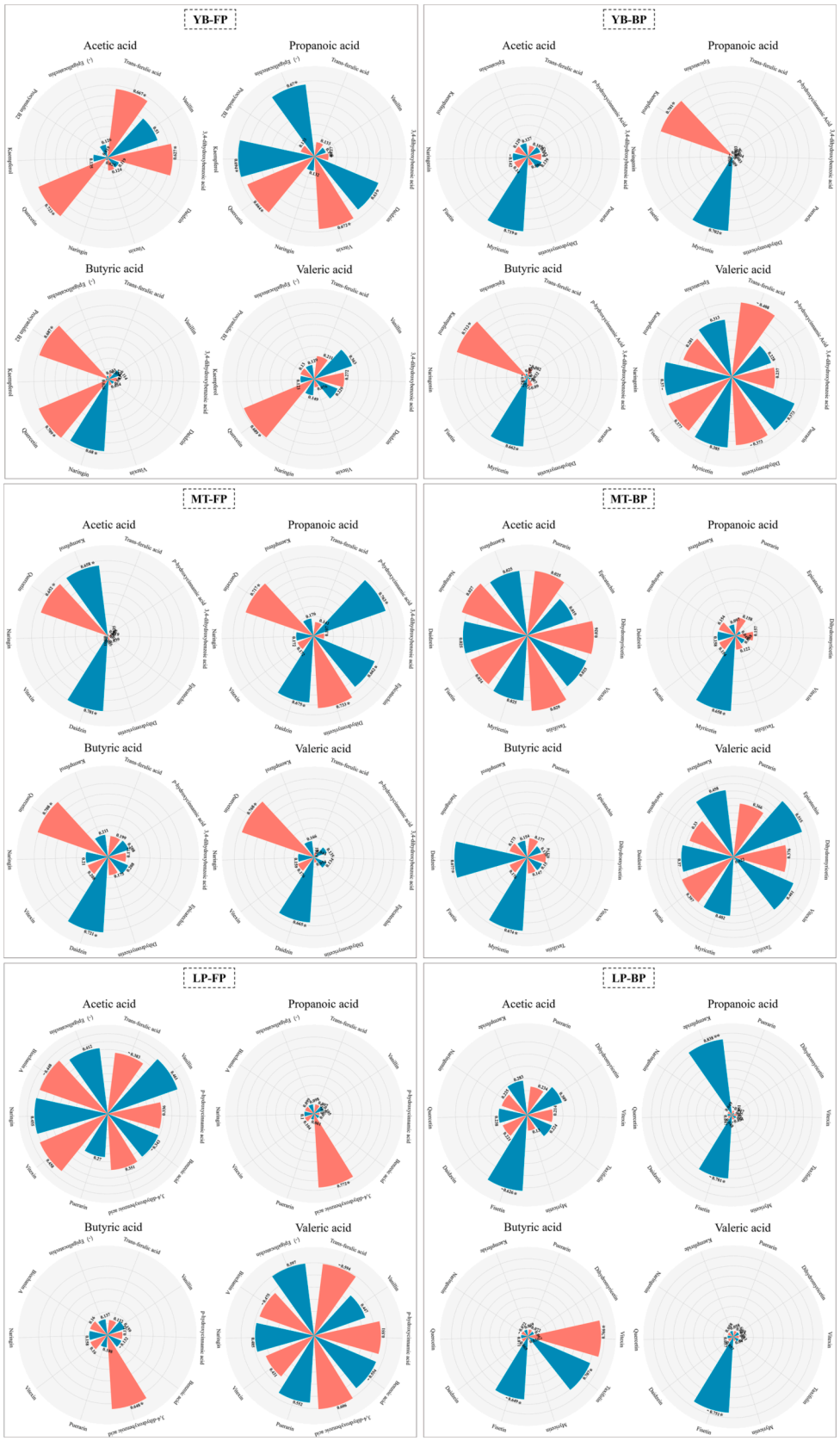

Fig. 5. Correlation analysis between SCFAs (acetic acid, propanoic acid, butyric acid, valeric acid) and polyphenols compounds. * Correlation was significant at $p < 0.05$. ** Correlation was extremely significant at $p < 0.01$. FP, Free polyphenols; BP, Bound polyphenols.

kaempferol and 3,4-dihydroxybenzoic acid showed a significant positive correlation with *Bifidobacterium*; and there was a significant positive correlation between daidzein and *Lactobacillus*. Due to its relatively high polarity, naringin remained in the jejunum, cecum, and colon for a relatively long time and was hydrolyzed by the microbiota to glycosides, regulating, *Lactobacillus*, *Clostridium*, *Enterococcus*, *Bacteroides*, and *Eubacterium brachy* (Wang et al., 2021); the content of daidzein was increased 4.4-fold in microplastic-induced Sprague-Dawley rats, and it possessed a positive link with f_*Oscillospiraceae* (p_*Firmicutes*), f_*Pasteurellaceae* (p_*Proteobacteria*), and f_*Veillonellaceae* (p_*Firmicutes*) (Zhao et al., 2023). The above analysis revealed that quercetin, daidzein, kaempferol, and naringin in distiller grains might be the key chemical compounds in improving the gut microbial community, implying the potential of distiller grains in regulating gut function.

Effect of the distiller's grain polyphenols on SCFAs

SCFAs are mainly produced by fermentation of dietary fiber by gut microbiota, which have essential roles in providing energy to cells, improving metabolic disorders, and maintaining homeostasis of the intestinal environment, especially acetic, propionic, and butyric acids (Zapata et al., 2022; Li et al., 2023). Acetic acid made up the main share of the total SCFAs, which are absorbed in the peripheral circulation and used as a source of energy (Li et al., 2023). In this study, the distiller's grain polyphenols promoted ($p < 0.05$) the production of SCFAs (Fig. 4). Free polyphenols of YB had the best promotion effect on acetic acid among all samples at simulating fermentation for 24 h. In the free polyphenols of YB, the acetic acid content (264.53 ± 5.91 mmol/L) was approximately 1.99 times higher than that of the control group (132.72 ± 6.86 mmol/L), and the promotion effect increased dose-dependently. Propionic and butyric acids are primarily used as energy sources by colon cells, which is important in maintaining homeostasis in the digestive system (Zapata et al., 2022). Compared with the control group, all samples promoted ($p < 0.05$) the production of propionic acid. Free polyphenols of three samples and bound polyphenols of MT and LP increased ($p < 0.05$) butyric acid content. In the bound polyphenols of LP, the butyric acid content (0.33 ± 0.02 mmol/L) was approximately 1.71 times higher than that of the control group (0.19 ± 0.02 mmol/L) at 24 h of simulated fermentation. In brief, the polyphenols in distiller grains promoted the production of the main SCFAs (acetic acid, propionic acid, butyric acid, valeric acid). Free polyphenols of YB and bound polyphenols of LP were better than other groups in promoting acetic acid and butyric acid, respectively.

SCFAs are closely related to the composition and abundance of the gut microbiota (Ahmad et al., 2020; Cheng et al., 2020). Compared to the control group, free polyphenols of LP promoted ($p < 0.05$) acetic acid production and decreased butyric acid content throughout the simulated fermentation phase. *Lactobacillus*, *Bifidobacterium*, *Ruminococcus*, *Prevotella*, *Bacteroides*, *Clostridium*, and *Streptococcus* were associated with acetic acid production. Acetic acid and butyric acid could be converted into each other by the action of the corresponding enzymes (Tang et al., 2021). All samples promoted ($p < 0.05$) the production of propionic acid. *Megasphaera* and *Faecalibacterium* can convert fermentable carbohydrates in the gut to propionic acid (Yu et al., 2019). Similar to the present study, cereal (sorghum and wheat) polyphenols increased the propionic acid content in an *in vitro* simulated colonic fermentation system (Gong et al., 2018).

The association analysis between SCFAs and polyphenol compounds is shown in Fig. 5. In bound polyphenols of LP and free polyphenols of MT and YB, quercetin showed positive correlations with SCFAs (acetic, propionic, butyric, valeric acids), especially significant ($p < 0.05$) positive correlations in free polyphenols of MT and YB. Positive correlations were found between myricetin, daidzein, kaempferol, and SCFAs. In free polyphenols of MT, daidzein and kaempferol were positively ($p < 0.05$) correlated with SCFAs (acetic acid, propionic acid, butyric acid, valeric acid) and acetic acid, respectively. In free polyphenols of YB, daidzein and kaempferol showed a positive ($p < 0.05$) correlation with propionic acid; in bound polyphenols of YB, myricetin and kaempferol were positively ($p < 0.05$) correlated with propionic acid and butyric acid; in bound polyphenols of MT, there was a positive ($p < 0.05$) correlation between myricetin and SCFAs (propionic acid, butyric acid). In addition, *trans*-ferulic acid, 3,4-dihydroxybenzoic acid, quercetin, and kaempferol showed a positive ($p < 0.05$) correlation with acetic acid in the free polyphenols of YB, which might be closely related to the increase in acetic acid content. In bound polyphenols of LP, there was a significant positive correlation between vitexin, taxifolin, and butyric acid, implying that vitexin and taxifolin promoted butyric acid production. The above analysis showed that both free and bound polyphenols from the three distillers' grains promoted the key SCFAs (acetic acid, propionic acid, butyric acid), while the effect varied among distillers' grains polyphenols. Free polyphenols of YB and bound polyphenols of LP were better than other groups in promoting acetic acid and butyric acid, respectively. Quercetin, myricetin, kaempferol, and daidzein were the key compounds affecting SCFAs.

Conclusion

Polyphenols from distiller's grain possessed antioxidant activity as well as the potential to improve the fecal microbiota community structure and the SCFAs production, attributing to the presence of quercetin, myricetin, kaempferol, and daidzein in the distiller's grain. The findings in this study will offer a valuable approach for transforming distiller's grain into functional food ingredients and promoting the high-value utilization of distiller's grain. It is crucial to explore the *in vivo* activities and effective applications of key polyphenolic compounds in further research.

CRediT authorship contribution statement

Jiang Zhong: Data curation, Methodology, Writing – original draft, Writing – review & editing. **Die Zhou:** . **Penggang Hu:** . **Yuxin Cheng:** Funding acquisition, Resources, Methodology. **Yongguang Huang:** Investigation, Funding acquisition, Methodology, Resources.

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.2023.101001.

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