Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

Polyphenol composition and antioxidant activity of fermentation combined with enzymatic hydrolysis modified *Astragalus membranaceus* stems

Na Liu[1,2,3], Xiaoping An[1,2,3*], Yuan Wang[1,2,3*], Jingwei Qi[1,2,3], Yang Jia[1,3], Xia Li[1,2,3], Zhalaga Zhang[1,2,3] and Xinnan Chen[1,2,3]

Abstract

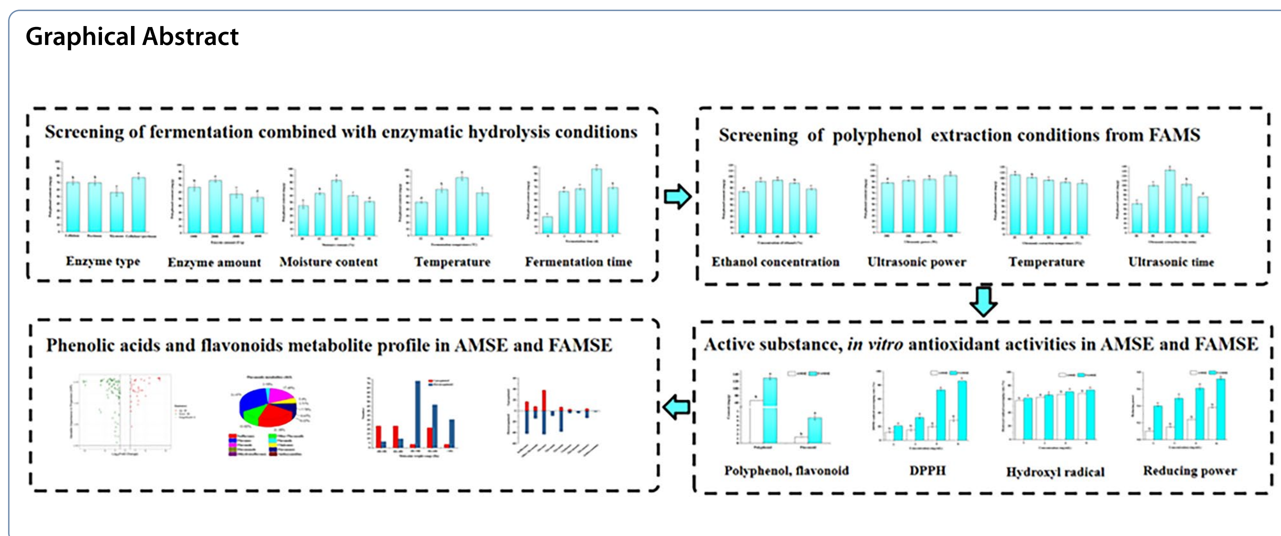
Astragalus membranaceus (AM) roots are a well-known homologous medicine and food in China. AM stems, which are discarded and not used effectively, also contain many active compounds and exhibit beneficial effects. It has the potential to be explored as antibiotic alternative. Fermentation combined with enzymatic hydrolysis (FEH) is an effective strategy for extracting polyphenol and improving the usage of AM stems. Therefore, in this study, the conditions of FEH and extraction for polyphenol in AM stems were screened. The antioxidant activity of extract from AM stems without and with FEH (AMSE and FAMSE) was evaluated. The metabolite profiles of phenolic acids and flavonoids in AMSE and FAMSE were characterized by ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC–ESI-MS/MS). The results showed that the highest polyphenol content from AM stems was obtained with cellulase and pectinase (1:1, 2000 U/g), moisture content 43%, fermentation temperature 30 °C, and fermentation time 7 days. Selected extraction conditions of polyphenol were ethanol concentration 50%, ultrasonic power 500 W, extraction temperature 35 °C, and extraction time 40 min. On this condition, compared with AMSE, the polyphenol and flavonoid contents in FAMSE were significantly higher. FAMSE exhibited stronger DPPH, hydroxyl radical scavenging rate and reducing power than AMSE. The relative content of 3-(4-hydroxyphenyl)-propionic acid, dihydroferulic acid, isoferulic acid, 4-hydroxybenzoic acid, 4-hydroxyphenyllactic acid, ferulic acid, vanillic acid, syringic acid, gentisic acid, sinapic acid, apigenin, tricin, acacetin, daidzein, genistein, formononetin, prunetin, pratensein, rhamnocitrin and galangin were significantly upregulated in FAMSE.

Keywords Fermentation, Enzymatic hydrolysis, *Astragalus membranaceus* stems, Polyphenol, Antioxidant activity

*Correspondence:
Xiaoping An
xiaoping_an@163.com
Yuan Wang
wangyuan@imau.edu.cn
Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Graphical Abstract



Introduction

Synthetic antibiotics have been used in farm animal production for decades to prevent and treat infectious diseases and improve animal productivity [1]. However, excessive use of antibiotics may lead to antibiotic residue in animal products and further hazards to human health [2, 3]. The need to have research on medicinal plants has been acknowledged worldwide by practitioners in order to find safe and effective antibiotic alternatives. Traditional Chinese Herbal Medicine plants, such as *Astragalus membranaceus* (AM), have been found that using as antibiotic replacements could increase growth performance, improve feed efficiency, and enhance antioxidant capacity and immunity of farm animals [4, 5]. AM is a perennial medicinal herb, whose roots is a well-known homologous medicine and food in China [6]. AM roots contains various active ingredients, such as polyphenol, flavonoids, polysaccharides, triterpenes, and saponins [7, 8], and has a broad range of bioactivities, including antioxidant, immune regulation, anti-inflammatory, anticancer and antivirus [9–12]. Several reports are available for antioxidant activities and immune functions of aerial parts of AM, such as stems and leaves [13–16]. Nayeem et al. found that methanolic extracts of AM stems exhibited analgesic and anti-inflammatory activity due to presence of polyphenol [17]. Thus, polyphenol of AM stems has the potential to be explored as a new antibiotic alternative.

Polyphenol are crucial secondary plant metabolites, which possess at least one aromatic ring with one or more hydroxyl substituents [18]. These compounds represent the most important group of natural antioxidants [19], which can reduce inflammation and ameliorate oxidative stress [20, 21]. Polyphenol can be categorized into two main subgroups: phenolic acids and flavonoids [22]. Cui et al. obtained two main flavonoids, isoquercitrin and astragalin, from AM stems with strong antioxidant activity [16]. However, due to plant cell wall recalcitrance, the extraction of AM stems polyphenol is limited [23]. It is suggested that fermentation is a useful strategy, which could effectively release the polyphenol from processing by-products of cereals, fruits, and vegetable oil [24]. Wang et al. observed an increase in polyphenol content of fermentation of rapeseed meals with mixed strains of *Bacillus subtilis* and *Saccharomyces cerevisiae* [25]. Qiao et al. reported that fermentation by *Lactobacillus plantarum* and/or *Enterococcus faecium* increased the production of polysaccharides, saponins, and flavonoid of AM roots [26]. During the fermentation, extracellular enzymes such as cellulase and pectinase were produced by microorganisms, which could rupture the herbal cell wall and release the bioactive ingredients inside [27]. Recently, fermentation combined with enzymatic hydrolysis (FEH) is regarded as a more promising method for the bioconversion of biomass or production active ingredients. Bei et al. found that compared to the microbial fermentation, the polyphenol contents of oats were increased by FEH [28]. Liu et al. proved that FEH enhanced the polyphenol content and antioxidant activities of aqueous solution of rice bran [29]. Moreover, our previous study also found that FEH with mix strain (*S. cerevisiae*, *B. subtilis*, and *L. plantarum* at a ratio of 1:1:1) and 500 U/g glucanase enhanced polyphenol contents and antioxidant activities of rice bran [30].

Thus, this study was conducted to screen conditions of FEH and extraction for polyphenol in AM stems, evaluate the in vitro antioxidant activity as well as phenolic acids and flavonoids metabolite profile of polyphenol in

AM stems extracts without and with FEH (AMSE and FAMSE) and provide a new antibiotic alternative rich in polyphenol from AM stems.

Materials and methods

Materials

AM stems, soybean meal, wheat bran, red dates powder, and molasses were purchased from the local market in Guyang, Baotou, Inner Mongolia, China. The stems of AM were air-dried, cut into slices, and used as the main substrate for FEH. *Bacillus subtilis* (CGMCC 1.0892), *Lactobacillus plantarum* (CGMCC No. 1.12934), and *Saccharomyces cerevisiae* (CGMCC No. 2.1190) were purchased from the China General Microbiological Culture Collection Centre (Beijing, China). Cellulase, pectinase, and xylanase were obtained from Beijing Solai Biotechnology Co., LTD. (Beijing, China). Other regents were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Screening of FEH conditions for AM stems

The AM stems is processed by FEH to release polyphenol. Based on previous studies, *B. subtilis* and *S. cerevisiae* were precultured in nutrient broth medium and malt extract medium at 35 °C and 30 °C in a rotary shaker (120 rpm) for 24 h, respectively. *L. plantarum* was precultured in MRS broth at 37 °C for 24 h. The compound probiotics inoculum was prepared by mixing the three inoculants (1.0×10^8 CFU/mL) at a ratio of 1:1:1. The AM stems (73%, w/w) were mixed with soybean meal (9%, w/w), wheat bran (9%, w/w), red dates powder (4.5%, w/w), and molasses (4.5%, w/w), and the mixture was inoculated with 1.0% of compound probiotics inoculum. After stirring evenly, a certain amount of distilled water was added. The effects of enzymatic type (cellulase, xylanase, pectinase, and cellulase + pectinase), enzyme amount (1000, 2000, 3000, and 4000 U/g), moisture content (20%, 33%, 43%, 50% and 55%, v/w), fermentation temperature (22 °C, 26 °C, 30 °C, and 32 °C) and fermentation time (3, 5, 7, and 9 d) on polyphenol content were investigated with a single-factor optimization experiments. The fermented products were dried at 45 °C and crushed to determine polyphenol content. And the polyphenol content of AM stems without fermentation or enzymatic hydrolysis (AMS) and AM stems with FEH under the optimum conditions (FAMS) were compared.

Screening of polyphenol extraction conditions from FAMS

The FAMS were prepared by the above optimum FEH conditions. Then, the ultrasonic-assisted ethanol extraction method was used to extract the polyphenol of FAMS. An ultrasonic cleaner (Branson 8510 Ultrasonic Cleaner, USA) was used. The ratio of ethanol to sample was 1:30. The effects of concentration of ethanol (40%, 50%, 60%, 70%, and 80%), ultrasonic power (200, 300, 400, and 500 w), ultrasonic extraction temperature (35 °C, 45 °C, 55 °C, 65 °C and 75 °C) and ultrasonic extraction time (20, 30, 40, 50 and 60 min) on polyphenol content were investigated with a single-factor optimization experiments. The extract was filtered, then the supernatant was centrifuged (3500 rpm, 15 min), concentrated and freeze-dried by a vacuum freeze-dryer (Scientz-100F). And the polyphenol and flavonoid contents of extracts of AMS and FAMS (AMSE and FAMSE) were determined.

The determination of polyphenol and flavonoid contents

The polyphenol contents were determined using previous methods [30] with gallic acid as the standard. The determination of flavonoid contents was conducted followed our previous method [31].

In vitro antioxidant activity of AMSE and FAMSE

The in vitro antioxidant activities AMSE and FAMSE were estimated by DPPH and hydroxyl radical scavenging activities as well as reducing power. DPPH radical scavenging activity and reducing power were determined by the method of Liu et al. [31]. The hydroxyl radical scavenging activity was determined with the method of Yin et al. [32].

Phenolic acids and flavonoids metabolite profile of polyphenol in AMSE and FAMSE

The phenolic acids and flavonoids metabolite profile of polyphenol in AMSE and FAMSE was analyzed by a UPLC–ESI-MS/MS system. The samples were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. The 100 mg of sample was dissolved with 1.2 mL 70% methanol solution, vortexed 30 s every 30 min for 6 times, and placed in a refrigerator at 4 °C overnight. Following centrifugation at 12,000 rpm for 10 min, the solution were filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China) before UPLC–MS/MS analysis. The HPLC and mass spectrum conditions were conducted with previous method [33]. Based on the self-built database MWDB (Metware Biotechnology Co., Ltd. Wuhan, China) and public database of metabolite information, the qualitative analysis of substance was performed using secondary mass spectrometry data. The quantification of metabolites was performed using multiple reaction monitoring (MRM) mode analysis [33]. Three replicates for AMSE and FAMSE were performed.

Statistical analysis

All experiments were performed in triplicate. For screening conditions of FEH and extraction for polyphenol in AM stems, one-way ANOVA followed

by Duncan's multiple range tests in SAS were used. The differences were accepted as significant at $P < 0.05$. The data are expressed as the mean ± standard deviation.

Metabolite data were $\log_2$-transformed for statistical analysis to improve normality and were normalized. Principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) were used to analyze the multivariate differences of metabolites by R software. The metabolites with a fold change (FC) ≥ 2 or ≤ 0.5 and a variable importance in projection (VIP) ≥ 1 was considered as significantly differential.

Results and discussion

Screening of FEH conditions for AM stems

Microbial fermentation is a traditional process for modifying traditional Chinese medicine. During fermentation, cell wall hydrolyzing performed by enzymes, such as cellulases, amylase, hemicelluloses, and pectinase, play critical roles in releasing bound polyphenol [27]. Park et al. found that the phenolic contents of AM was significantly increased after *Lactiplantibacillus plantarum* fermentation [34]. Moreover, Wang et al. reported that complex enzymatic hydrolysis (cellulase, xylanase, hemicellulose and β-glucosidase) after fermentation increased the contents of phenolics, flavonoids, quercetin, and kaempferol in guava leaves tea [35]. Bei et al. found that the polyphenol contents of oats treated by FEH was higher than that by microbial fermentation [28]. It is indicated that FEH might be a more promising method for releasing bound polyphenol. Therefore, the effects of enzymatic type and enzyme amount were investigated. As presented in Fig. 1A, compared with xylanase, the usage of cellulase and pectinase alone significantly increased polyphenol content ($P < 0.05$). The highest polyphenol content was achieved using cellulase + pectinase (1:1). With increasing enzyme amount, the polyphenol content increased and then decreased, and the highest polyphenol content was reached with adding 2000 U/g enzymes (Fig. 1B). These results indicated that adding 2000 U/g cellulase + pectinase could hydrolyze the cell wall structure of AM stems and release polyphenol. Cellulases refer to a multi-enzyme mixture and have been reported as an effective tool for hydrolyzing plant cell walls [28]. Pectinase is also a cell wall degrading enzyme which degrades pectic polymers into galacturonic acid [36]. Consistent with our results, Siddiq et al. reported that pectinase and cellulase increased the phenolic contents by up to 6.4 times compared to the untreated blueberry juice [37].

Moisture content, fermentation temperature, and fermentation time are essential factors for metabolite production during fermentation [31]. The suitable moisture content was found to be 43%, which yielded the highest polyphenol content (83.09 mg/g) (Fig. 1C). As shown in Fig. 1D, the polyphenol content increased with increasing fermentation temperature, and the maximum concentration was recorded at 30 °C. Then, at higher fermentation temperature, the polyphenol content decreased. The polyphenol contents significantly increased from 3 to 7 days, reached maximum values of 97.39 mg/g on the day 7, then declined in the subsequent culture (Fig. 1E). Our results suggested that the suitable FEH conditions of AM stems were as follows: enzyme type cellulase + pectinase (1:1), enzyme amount 2000 U/g, moisture content 43%, fermentation temperature 30 °C, and fermentation time 7 days. Under the suitable FEH conditions, the polyphenol content of AM stems was 85.48 mg/g, which was 2.43 times higher than that without FEH treatment (24.93 mg/g).

Screening of polyphenol extraction conditions from FAMS

Ultrasonic-assisted extraction technique is a proven green bio-refining technology [38], which can destroy cell walls to promote solute diffusion and increase the extraction efficiency of target compounds [39, 40]. Iftikhar et al. found that ultrasonic-assisted extraction resulted in a higher yield of rye bran's phenolic and flavonoid compounds than the conventional extraction technique [41]. Therefore, the ultrasonic-assisted extraction was used in this study to extract polyphenol from FAMS. Ethanol is generally recognized as a safe solvent and has been used to extract polyphenol. The ethanol concentration affects the target components' solubility and extraction yield [16]. As shown in Fig. 2A, the polyphenol content was significantly higher when the ethanol concentration was 50% and 60% ($P < 0.05$), however, there was no remarkable difference between 50 and 60%. Therefore, ethanol concentration of 50% was screened for the subsequent extraction test. With ultrasonic power increasing from 200 to 500 W, the polyphenol content increased (Fig. 2B), which was agreed with Ma et al., who confirmed the positive effects of increasing ultrasonic power on polyphenol content [42]. Thus, the ultrasonic power 500 W was chosen to extract polyphenol from FAMS. It is found that appropriate heat treatment increases the solubility of polyphenol in the solution [43]. The effects of ultrasonic extraction temperature on the polyphenol content are presented in Fig. 2C. It was found that highest polyphenol content was achieved at 35 °C. Furthermore, longer extraction time may accelerate the absorption of solvent, soften the plant tissues, weaken the cell wall integrity, and thus enhanced ingredient solubility [43]. However, excessive extraction time may oxidize polyphenol, reduce extraction efficiency, and waste time [16]. In this study,

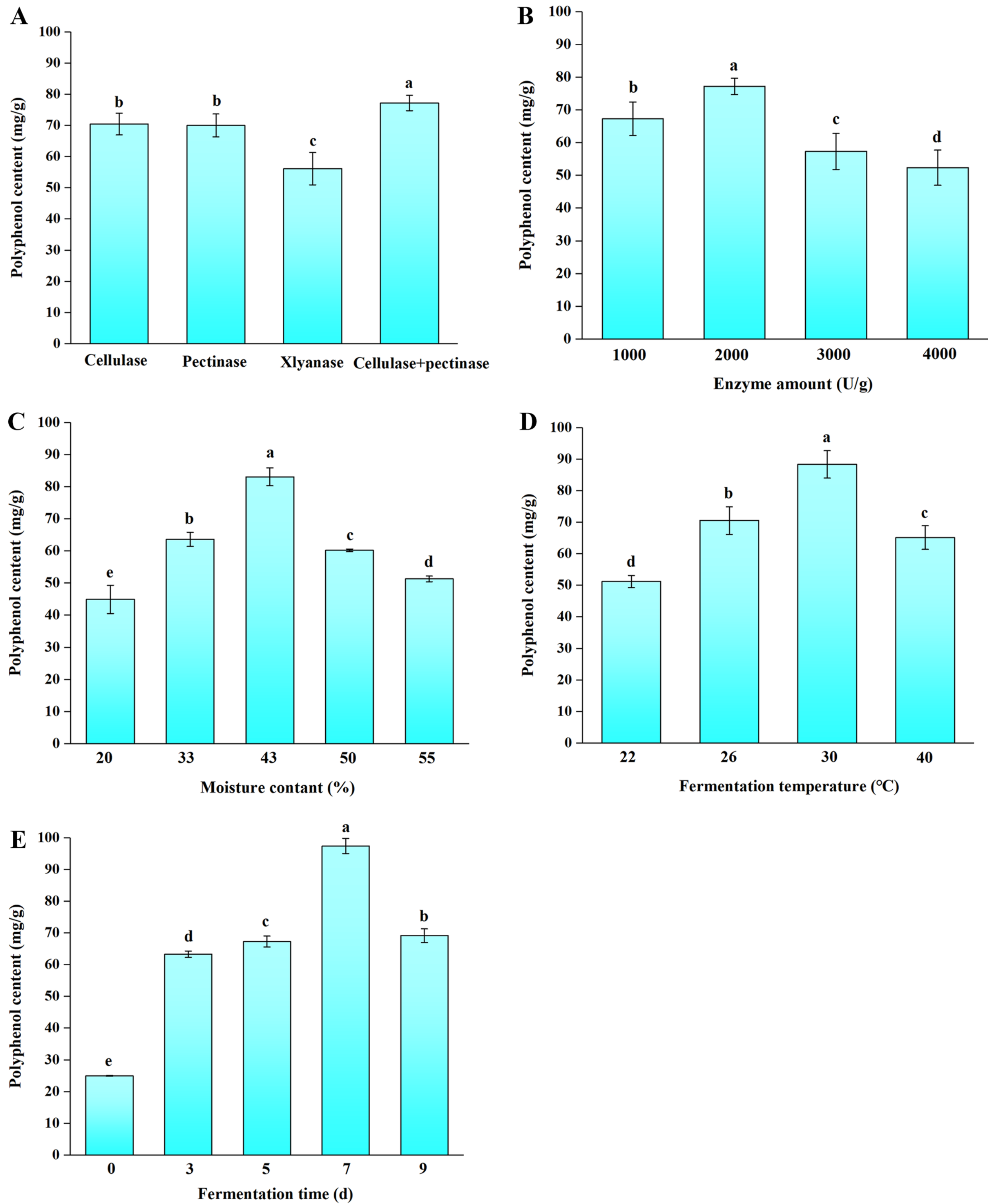


Fig. 1 Effects of FEH conditions on the polyphenol content of AM stems. **A** Enzyme type. **B** Enzyme amount. **C** Moisture content. **D** Fermentation temperature. **E** Fermentation time. Different lowercase letters in bars represent significant differences ($P < 0.05$)

Fig. 2 Effects of ultrasonic-assisted ethanol extraction conditions on the polyphenol content of FAMS. **A** Concentration of ethanol. **B** Ultrasonic power. **C** Ultrasonic extraction temperature. **D** Ultrasonic extraction time

the polyphenol content fluctuated between 62.03 and 132.80 mg/g with 20–60 min extraction time (Fig. 2D). It can be seen that extraction time had a significant effect on polyphenol content of FAMS ($P<0.05$). The polyphenol content was gradually increased up to 40 min of extraction and then started falling. Our results indicated that the suitable extraction conditions of polyphenol from FAMS were as follows: ethanol concentration 50%, ultrasonic power 500 W, extraction temperature 35 °C, and extraction time 40 min.

Polyphenol and flavonoid contents in AMSE and FAMSE

Figure 3 shows the polyphenol and flavonoid contents in AMSE and FAMSE. Under the optimal FEH and extraction conditions, the polyphenol content in AMSE and FAMSE were 66.22 mg/g and 129.34 mg/g, respectively. And the flavonoid contents

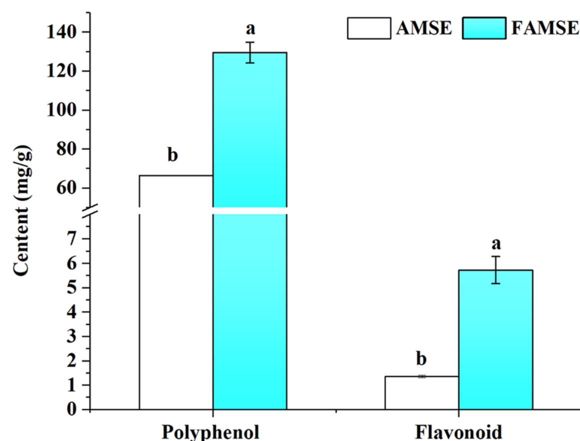


Fig. 3 Polyphenol and flavonoid contents in AMSE and FAMSE

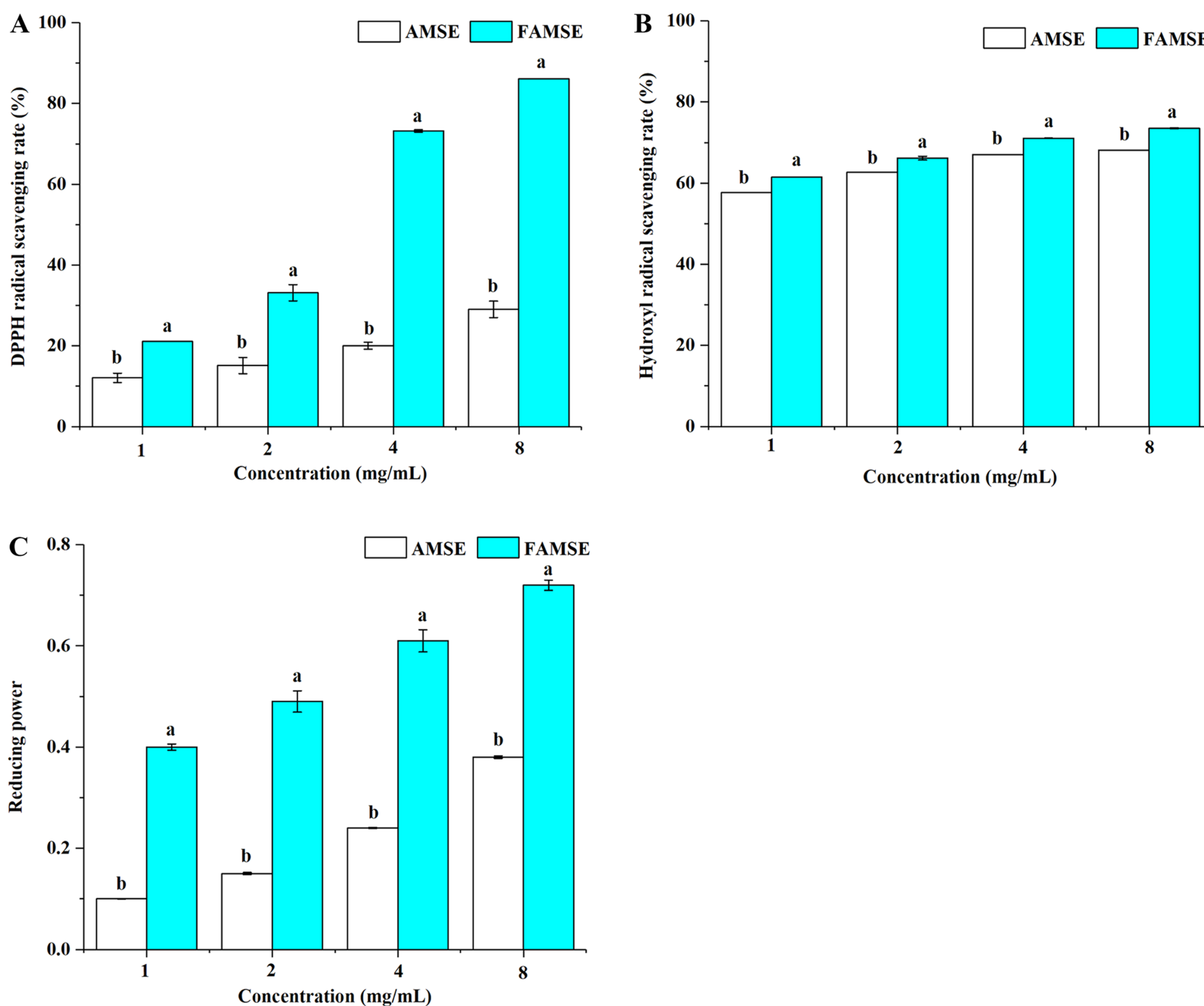


Fig. 4 Antioxidant activities of AMSE and FAMSE. **A** DPPH radical scavenging rate. **B** Hydroxyl radical scavenging rate. **C** Reducing power. Different lowercase letters in bars represent significant differences between AMSE and FAMSE ($P < 0.05$)

in AMSE and FAMSE were 1.35 mg/g and 5.72 mg/g, respectively. Compared with AMSE, polyphenol and flavonoid contents in FAMSE was significantly higher by 92.46% and 323.70%, respectively ($P < 0.05$). A study investigated the effects of fermentation by *Lactiplantibacillus plantarum* on AM, and found that fermented AM had higher phenolic content [34]. It was also reported that hydrolysis with carbohydrase enzymes, such as pectinases and cellulases, at suitable conditions effectively released the insoluble phenolics [44]. More importantly, it was found that the flavonoid content in AM was significantly increased with *Lactobacillus pentosus Stm* solid-state fermentation combined with the addition of xylanase, cellulase, and pectinase [45]. Consistent with previous research findings, FSH treatment increased polyphenol and flavonoid contents in AM stems in this study. The release of polyphenol and flavonoid contents may be due to both the utilization of cell wall polysaccharides by probiotics and hydrolyzation of ester bond between phenolics and cell wall components by carbohydrase [29, 44].

In vitro *antioxidant activities of AMSE and FAMSE*

Reactive oxygen (ROS) and reactive nitrogen (RNS) species are produced during the metabolism in the animal body. Over production of ROS and RNS may lead to physiological imbalances, which result in chronic inflammation and numerous diseases [46]. Natural antioxidants from plants can protect animal body from adverse free radicals and prevent many diseases. Previous studies have reported that polyphenol are natural antioxidants against ROS and RNS species [47]. The improvement of antioxidant capacity was strongly

associated with increased polyphenol content [44]. It was found in this study that FSH treatment increased polyphenol and flavonoid contents in AM stems. Therefore, the DPPH and hydroxyl radical scavenging rate as well as reducing power of AMSE and FAMSE were tested (Fig. 4). As shown in Fig. 4A–C, both AMSE and FAMSE exhibited antioxidant activity in the test concentration. Notably, the DPPH and hydroxyl radical scavenging rate as well as reducing power of FAMSE were significantly higher than those of AMSE ($P < 0.05$). Consistently, Kuo et al. revealed that fermentation improved the phenolic content and scavenging activity against DPPH and ABTS of *Chenopodium formosanum* Koidz [48]. Zhang et al. found FEH enhanced phenolic compound content and DPPH radical scavenging rate of quinoa [49].

Phenolic acids metabolite profile of polyphenol in AMSE and FAMSE

Phenolic acids are hydroxyl derivatives of aromatic carboxylic acids, which are powerful natural antioxidants [49]. A total of 179 phenolic acids were identified in AMSE and FAMSE. In the PCA score plot (Fig. S1A), three principal components (PC1, PC2, and PC3) were 77.94%, 6.98%, and 5.93%, respectively. In verification chart of OPLS-DA model (Fig. S1B), The R^2X, R^2Y and Q^2 were 0.862, 1 and 0.995, respectively. It is demonstrated that the model was stable and reliable by the Q^2 values exceeded 0.9. The PCA and OPLS-DA analysis showed that the two groups were well-separated, suggesting that FSH significantly affected phenolic acids metabolite profile of AM stems. There were 119 significantly different phenolic acids metabolites between AMSE and FAMSE, among which 39 were upregulated and 80 were downregulated (Fig. S1C).

The differential phenolic acids metabolites between AMSE and FAMSE are shown in Table 1. In comparison with AMSE, the relative contents of 3-(4-hydroxyphenyl)-propionic acid, dihydroferulic acid (DHFA), isoferulic acid (IFA), 4-hydroxybenzoic acid, 2-hydroxy-3-phenylpropanoic acid, moracin Y, 4-hydroxyphenyllactic acid, ferulic acid (FA), vanillic acid, syringic acid, 2,3-dihydroxybenzoic acid, gentisic acid, benzoic acid and sinapic acid in FAMSE increased, while the relative contents of 4-*O*-glucosyl-4-hydroxybenzoic acid, glucosyloxybenzoic acid, salidroside, 1-*O*-salicyloyl-β-D-glucose, 1-(4-hydroxybenzoyl)glucose, 6′-*O*-feruloyl-D-sucrose, 5-hydroxyisovanillic acid, 4-hydroxybenzoic acid glucosyl xyloside, 3,4-dihydroxybenzaldehyde-xylose-glucoside, benzoylmalic acid, dihydroferulic acid glucoside and glucosyringic acid decreased. It is reported that 4-*O*-glucosyl-4-hydroxybenzoic acid, glucosyloxybenzoic acid, 1-*O*-salicyloyl-β-D-glucose, 1-(4-hydroxybenzoyl) glucose, 5-hydroxyisovanillic acid, 4-hydroxybenzoic acid glucosyl xyloside, 3,4-dihydroxybenzaldehyde-xylose-glucoside, benzoylmalic acid and glucosyringic acid could be degraded into other compounds, such as 4-hydroxybenzoic acid, vanillic acid, syringic acid, 2,3-dihydroxybenzoic acid, gentisic acid and benzoic acid, by enzymes (cellulase, pectinase et al.) produced by microbial metabolism or added exogenous. 6′-*O*-feruloyl-D-sucrose, dihydroferulic acid glucoside also could be metabolized into other compounds, such as DHFA, IFA and FA. And salidroside could probably degraded into tyrosol and glucose by β-D-glucosidase [49].

Phenolic acids can be further divided into two distinctive groups: hydroxycinnamic (basic skeleton C6-C3) acids and hydroxybenzoic (basic skeleton C6-C1) [50]. Ferulic, caffeic, p-coumaric, and sinapic acids are major representative substances of hydroxycinnamic acids [51]. As the major hydroxycinnamic acid, FA is widely known for its antioxidant, antimicrobial, and anti-inflammatory properties [52]. In this study, the relative content of FA (3.93-fold) in FAMSE was higher compared to AMSE (Table 1). FA can be metabolized to DHFA and IFA [53]. It is reported that DHFA is a better antioxidant and anti-inflammatory than FA [54, 55]. A study by Lee et al. revealed that DHFA obtained from fermented rice bran extract reversed the reduction of viability of PC12 cells caused by H_2O_2 and enhanced the transcription levels of antioxidant genes [56]. The IFA could be used for treating several inflammatory diseases, clearance of ROS, elimination of viral infections, and alleviation of metabolic diseases and specific cancers [57]. Furthermore, esters form of FA, methyl ferulate, and ethyl ferulate, were proven to more efficiently prevent lipid oxidation [58, 59]. As shown in Table 1, the relative contents of DHFA, IFA, ethyl ferulate and ferulic acid methyl ester in FAMSE were upregulated by 104.45-, 3.34-, 13.65- and 6.21-fold, respectively. Additionally, the relative contents of sinapic acid were increased by 1.34-fold. Sinapic acid has been reported against various pathological conditions, such as oxidative stress, inflammation, infections, and cancer [60].

The family of hydroxybenzoic acids occurs naturally in plants and is the simplest class of natural antioxidants [21]. As a type of hydroxybenzoic acids, 4-hydroxybenzoic acid is a valuable aromatic compound used as raw material for producing liquid crystal polymers and paraben, which shows the higher antioxidant activity compared with its esters [61]. Vanillic acid is an oxidized form of vanillin with pleasant creamy odor and antioxidative activity [62]. Syringic acid is a high bioavailability and low toxicity polyphenol, which can be administered as a preventative measure against

Table 1 Relative content of part of phenolic acids differential metabolites in AMSE and FAMSE

Compounds	Relative content		VIP	FC	Type
	AMSE	FAMSE			
3-(4-Hydroxyphenyl)-propionic acid	3.37E+05	3.85E+07	1.16	114.37	Up
Dihydroferulic acid	1.37E+05	1.42E+07	1.12	104.13	Up
Isoferulic acid	3.29E+06	1.10E+07	1.13	3.34	Up
4-Hydroxybenzoic acid	7.79E+06	2.89E+07	1.15	3.70	Up
2-Hydroxy-3-phenylpropanoic acid	4.31E+05	3.69E+07	1.16	85.78	Up
Moracin Y	9.70E+05	2.67E+07	1.16	27.58	Up
4-Hydroxyphenyllactic acid	6.26E+04	6.49E+06	1.16	103.66	Up
Methyl 2,4-dihydroxyphenylacetate	6.04E+04	6.31E+06	1.16	104.45	Up
2-Hydroxy-3-(4-Hydroxyphenyl)propanoic acid	6.07E+04	6.57E+06	1.16	108.17	Up
3,4-Dimethoxyphenyl acetic acid	1.41E+04	1.59E+06	1.15	112.86	Up
Ferulic acid	2.26E+06	8.89E+06	1.15	3.93	Up
Vanillic acid	1.28E+06	3.21E+06	1.16	2.51	Up
2,3-Dihydroxybenzoic acid	1.64E+05	4.43E+06	1.15	26.93	Up
Syringic acid	3.73E+05	1.66E+06	1.16	4.45	Up
Methyl coumalate	4.27E+05	1.35E+06	1.13	3.36	Up
2,5-Dihydroxybenzoic acid; gentisic acid	3.64E+05	9.20E+05	1.14	2.53	Up
Benzoic acid	2.61E+05	7.85E+05	1.13	3.00	Up
Sinapic acid	6.54E+04	1.65E+05	1.05	1.34	Up
Ethyl ferulate	5.81E+04	7.93E+05	1.15	13.65	Up
Ferulic acid methyl ester	7.34E+04	4.56E+05	1.13	6.21	Up
Antiarol; 3,4,5-trimethoxyphenol	1.82E+04	3.06E+05	1.12	16.80	Up
Eudesmic acid (3,4,5-trimethoxybenzoic acid)	4.32E+04	3.03E+05	1.13	7.03	Up
Vnilloylmalic acid	7.30E+04	3.12E+05	1.13	4.27	Up
Pyrocatechol	1.15E+04	1.14E+05	1.13	9.94	Up
4-*O*-Glucosyl-4-hydroxybenzoic acid	3.87E+07	1.51E+05	1.15	0.00	Down
Glucosyloxybenzoic acid	3.49E+07	1.19E+05	1.15	0.00	Down
Salidroside	3.35E+07	1.64E+05	1.15	0.00	Down
1-*O*-Salicyloyl-β-D-glucose	3.54E+07	1.18E+05	1.15	0.00	Down
1-(4-Hydroxybenzoyl) glucose	6.17E+06	2.73E+04	1.14	0.00	Down
6′-*O*-Feruloyl-D-sucrose	1.05E+06	8.68E+04	1.15	0.08	Down
5-hydroxyisovanillic acid	1.25E+06	4.47E+04	1.14	0.04	Down
Phenylpropionic acid-*O*-β-D-glucopyranoside	1.97E+06	5.96E+04	1.08	0.03	Down
4-Hydroxybenzoic acid glucosyl xyloside	2.35E+07	2.94E+06	1.16	0.12	Down
3,4-dihydroxybenzaldehyde-xylose-glucoside	2.05E+07	2.56E+06	1.16	0.13	Down
Benzoylmalic acid	1.80E+07	7.85E+06	1.07	0.44	Down
Dihydroferulic acid glucoside	1.29E+06	1.73E+05	1.15	0.13	Down
Glucosyringic acid	3.22E+06	5.98E+05	1.12	0.19	Down
5-(2-Hydroxyethyl)-2-*O*-glucosylphenol	2.06E+06	7.09E+05	1.13	0.34	Down
Dihydroxybenzoyl xyloside	1.34E+06	6.43E+05	1.13	0.48	Down
Ferulic β-glucoside	3.94E+05	1.89E+05	1.12	0.48	Down
4-*O*-β-D-Glucopyranosylferulic acid	2.50E+05	6.62E+04	1.03	0.26	Down
4-Hydroxybenzoyl acetyl glucoside	6.75E+05	3.06E+05	1.14	0.45	Down
Sinapyl alcohol	1.75E+05	1.75E+04	1.11	0.10	Down
Methyl syringate	2.50E+05	9.08E+04	1.14	0.36	Down
Methyl 3-(3-hydroxy-4-methoxyphenyl) propanoate	1.28E+05	2.01E+04	1.13	0.16	Down

a range of diseases due to its antioxidant activity [63]. Similarly, many studies have been published about the antioxidant activity of gentisic acid, which have proposed its potential use in treating diseases [64]. As shown in Table 1, the relative contents of 4-hydroxybenzoic acid (3.70-fold), vanillic acid (2.51-fold), syringic acid (4.45-fold), 2,3-dihydroxybenzoic acid (26.93-fold), gentisic acid (2.53-fold) and benzoic acid (3.00-fold) in FAMSE significantly accumulated. Consistent with our results, Degrain et al. revealed that 2,5-dihydroxybenzoic acid was the predominant phenolic acid in the *L. plantarum*-fermented nightshade leaves, and *L. plantarum* fermentation improved the bioavailability of vanillic acid of nightshade leaves [65]. Darwesh et al. demonstrated that contents of *p*-hydroxybenzoic acid, gentisic acid, and syringic acid increased in *Saccharomyces cerevisiae*-fermented cinnamon [66]. Azkia et al. found that fermentation enhanced vanillic acid and vanillin levels in black glutinous rice [67]. In addition, 3-(4-hydroxyphenyl)-propionic acid (HPPA) and 4-hydroxyphenyllactic acid increased in FAMSE in this study. It is reported that 4-hydroxyphenyllactic acid can be produced by many lactic acid bacteria [68]. HPPA was a major microbial metabolite of A-type procyanidin, and inhibited suppressing effects on cellular oxidative stress and inflammation [69, 70].

Our results revealed that FEH changed phenolic acids metabolite profile of AM stems. The relative content of HPPA, DHFA, IFA, 4-hydroxybenzoic acid, 4-hydroxyphenyllactic acid, FA, vanillic acid, syringic acid, gentisic acid, and sinapic acid were significantly increased in FAMSE, which may be the reason for its enhanced antioxidant activity.

Flavonoids metabolite profile of polyphenol in AMSE and FAMSE

Flavonoids is an essential group of naturally occurring polyphenol compounds with basic structures consist of C6-C3-C6 rings. According to the substitution patterns, flavonoids could be categorized into a series of subclass, isoflavones, flavones, flavonols, flavanones, flavanols, and anthocyanins [71]. In this study, a total number of 463 flavonoids were identified in AMSE and FAMSE. Isoflavones, flavones, and flavonols were the main subclasses of flavonoids, which account for more than 70.84% of flavonoids in AMSE and FAMSE (Fig. 5). AMS and FAMS were separated in Fig. S2A, indicating that FEH had a substantial effect on flavonoids metabolite profile of AM stems. As shown in Fig. S2B, the R^2X, R^2Y and Q^2 were 0.845, 1 and 0.994, respectively, demonstrating that the OPLS-DA model was stable and reliable and could be applied to further screen differential flavonoids metabolites. There were 245 significantly differential flavonoids metabolites between AMSE and FAMSE (77 upregulated, 168 down-regulated) (Fig.S2C).

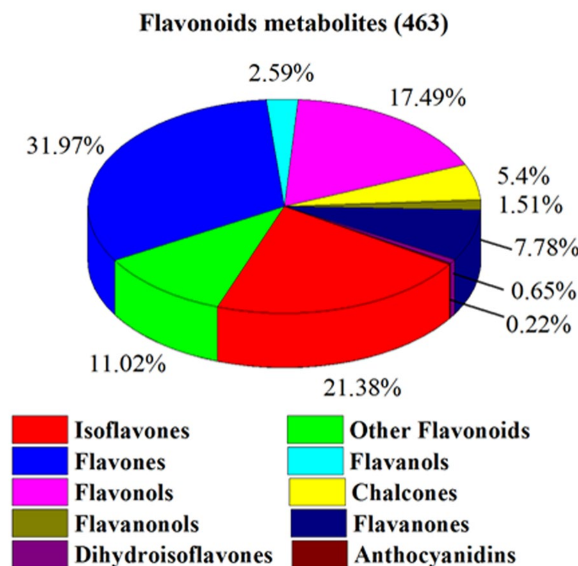


Fig. 5 Flavonoids classification chart according to subgroups

The differential flavonoids metabolites between AMSE and FAMSE were mainly classified into three subgroups, isoflavones, flavones, and flavonols, which account for 75.51% of the total differential flavonoids metabolites (Fig. 6A). As shown in Fig. 6B, most of the down-regulated flavonoids metabolites in FAMSE have molecular weights greater than 400 Da. In contrast, the molecular weights of upregulated flavonoids metabolites ranged in 200–400 Da and 500–600 Da.

Flavones is the most common flavonoids subclass, found in herbs and vegetables [21]. Apigenin, tricin and acacetin are the representative compounds of flavones. Apigenin is a polyphenolic flavone with a low molecular weight, responsible for antioxidant potential and chelating redox-active metals [72]. Tricin typically accumulates in the leaves and stems of herbaceous and cereal plants. It exists as free and derivative form, showing potential pharmaceutical applications due to its low toxicity and antioxidative activity [73]. Moreover, acacetin and diosmin have been proven to exhibit extensive biological capabilities, including antioxidative and anti-inflammatory properties [74, 75]. In this study, higher relatively contents of apigenin, tricin, acacetin, diosmin, isoschaftoside were observed in FAMSE (Table 2). Consistent with our findings, a previous study reported that the concentration of apigenin and tricin in brown juice from alfalfa were increased after lactic acid bacteria-fermentation [76]. It is revealed that the hydroxylation of the A-ring of flavonoids, especially for 5- and 7-hydroxylations, is beneficial for enhancement of antioxidant

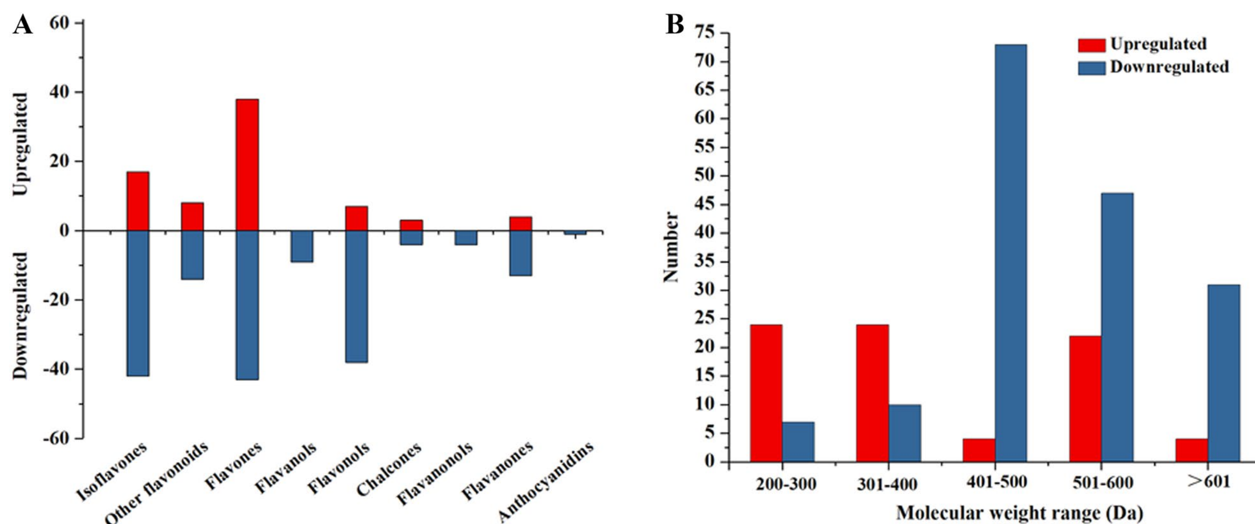


Fig. 6 Classification (**A**) and molecular weight range (**B**) of differential flavonoids metabolites between AMSE and FAMSE

activity [77]. In this study, relatively contents of 6,7-dihydroxyflavone and 3′,4′,7-trihydroxyflavone were higher in in FAMSE. Moreover, relatively contents of 5,6,7-tetrahydroxy-8-methoxyflavone, 5,7,3′,5′-tetrahydroxy-6-methylfavanone, apigenin-6-C-(2″-glucosyl) arabinoside and tricin-7-*O*-(2″-*O*-rhamnosyl) galacturonide were increased. As the major bioactive constituents of AM, methoxylated flavonoids and their glycosides have been proven to have superior antioxidant activity [78].

Isoflavones are flavones with a B ring attached to C3 instead of C2, commonly found in leguminous plants [79]. Daidzein and genistein are the most omnipresent isoflavones, which have similar biological activity and redox behavior [79]. The antioxidative activity of formononetin [80], prunetin [81, 82] and pratensein [83] had been confirmed. As shown in Table 3, the relative contents of daidzein (105.46-fold), genistein (29.38-fold), formononetin (2.03-fold), prunetin (2.80-fold), pratensein (2.96-fold), 4′,6,7-trihydroxyisoflavone (42.69-fold) and isoluteolin (25.40-fold) in FAMSE were significantly increased compared to AMSE. A report confirmed that isoflavonoid glycosides were degraded into aglycones when the AM extract was fermented, for instance, formononetin-glycosides into formononetin [84].

Flavonols, the most commonly occurring group of flavonoids, are flavones hydroxylated in C3, including kaempferol, quercetin, and isorhamnetin [21]. In this study, most relative contents of kaempferol glycosides were down-regulated in FAMSE, while the relative contents of rhamnocitrin and galangin were significantly upregulated (Table 4). It is suggested that during the FEH process, glycosidic bonds in AM stems were presumably hydrolyzed, thus leading to a decline in kaempferol glycosides. Rhamnocitrin has been reported to have antioxidant, anti-inflammatory, and antitumor activities [85]. Galangin is a potent antioxidant primarily derived from different medicinal herbs, which could alleviate oxidative stress and increase immune function [86].

Altogether, the flavonoids metabolite profile of AM stems was altered after FEH, and the relative content of apigenin, tricin, acacetin, daidzein, genistein, formononetin, prunetin, pratensein, rhamnocitrin and galangin were upregulated in FAMSE. Flavonoids are mostly glycosides and not as aglycones in plants [87]. Microbial fermentation could promote flavonoids

Table 2 Relative content of part of differential flavones metabolites in AMSE and FAMSE

Compounds	Relative content		VIP	FC	Type
	AMSE	FAMSE			
Apigenin; 4′,5,7-trihydroxyflavone	5.08E+05	1.41E+07	1.16	27.83	Up
6,7-Dihydroxyflavone	1.18E+05	1.29E+07	1.16	109.19	Up
3′,4′,7-Trihydroxyflavone	3.97E+05	1.12E+07	1.16	28.26	Up
5,6,7-Tetrahydroxy-8-methoxyflavone	3.60E+06	1.05E+07	1.15	2.92	Up
5,7,3′,5′-tetrahydroxy-6-methylfavanone	3.93E+06	1.03E+07	1.13	2.63	Up
Apigenin-6-C-(2″-glucosyl) arabinoside	8.61E+04	2.85E+06	1.16	33.1	Up
Apigenin-8-C-(2″-glucosyl) arabinoside	2.78E+04	1.00E+06	1.15	36.18	Up
Apigenin-7-*O*-rutinoside (isorhoifolin)	1.84E+04	1.07E+05	1.15	5.78	Up
Tricin (5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone)	3.65E+04	3.92E+06	1.16	107.25	UP
Tricin-4′-*O*-(guaiacylglycerol) ether	6.93E+04	3.25E+06	1.15	46.96	Up
Tricin-7-*O*-(2″-*O*-rhamnosyl) galacturonide	1.55E+04	2.10E+06	1.15	135.76	Up
Tricin-7-*O*-guaiacylglycerol	6.18E+04	2.73E+06	1.14	44.14	Up
Tricin-5-*O*-guaiacylglycerol	6.37E+04	2.72E+06	1.15	42.7	Up
8-Methoxyapigenin	3.62E+06	9.66E+06	1.14	2.67	Up
4′,5,7-Trihydroxy-3′,6-dimethoxyflavone (jaceosidin)	7.01E+05	2.16E+06	1.15	3.08	Up
Isoschaftoside	5.36E+04	1.90E+06	1.16	35.39	Up
Acacetin	5.70E+05	1.87E+06	1.16	3.28	Up
Diosmin	3.71E+04	1.41E+05	1.11	3.8	Up
Chrysoeriol-7-*O*-(6″-malonyl) glucoside	3.73E+07	2.53E+06	1.16	0.07	Down
Acacetin-7-*O*-(6″-malonyl) glucoside	2.34E+07	1.70E+06	1.16	0.07	Down
Hispidulin-7-*O*-glucoside (homoplantaginin)	1.44E+07	1.55E+06	1.16	0.11	Down
Diosmetin-7-*O*-galactoside	1.40E+07	1.28E+06	1.15	0.09	Down
Luteolin-7-*O*-(6″-malonyl) glucoside	6.46E+06	9.90E+05	1.16	0.15	Down
Apigenin-4′-*O*-glucoside	5.38E+06	8.04E+05	1.15	0.15	Down
Apigenin glucosyl malonyl glucoside	3.33E+06	6.68E+05	1.14	0.2	Down
Tricetin-5-*O*-(6″-malonyl) glucoside	3.16E+06	2.57E+05	1.16	0.08	Down
Chrysoeriol-7-*O*-glucoside	2.20E+06	2.29E+05	1.15	0.1	Down
Luteolin-7-*O*-(6″-malonyl) glucoside	9.01E+06	9.01E+06	1.16	0.08	Down
Chrysoeriol-7-*O*-(6″-acetyl) glucoside	8.89E+06	8.89E+06	1.16	0.08	Down
5,6,3′,4′-Tetrahydroxy-3,7-dimethoxyflavone	5.38E+05	8.28E+04	1.16	0.15	Down
Acacetin-7-*O*-rutinoside (linarin)	6.88E+05	6.53E+04	1.15	0.09	Down
Chrysoeriol-7-*O*-(6″-acetyl) glucoside	7.76E+05	5.87E+04	1.15	0.08	Down
Chrysoeriol-7,4′-di-*O*-glucoside	4.25E+05	4.42E+04	1.11	0.1	Down
Tricin-5-*O*-(6′-O-malonyl) glucoside	2.84E+05	4.20E+04	1.13	0.15	Down
Formononetin 7-*O*-Glucoside-6″-malonate	7.35E+05	1.65E+05	1.12	0.22	Down

glycosides converted to aglycones, which are more bioactive, easily absorbed in the small intestine, and have more potent antioxidant activity [88, 89]. The combination with enzymatic hydrolysis with microbial fermentation could boost the hydrolysis of sugar moieties, expedite the extraction of flavonoids, thus improve the antioxidant activities [87].

Conclusion

The suitable FEH conditions for polyphenol from AM stems were enzyme type cellulase and pectinase, enzyme amount 2000 U/g, moisture content 43%, fermentation temperature 30 °C, and fermentation time 7 days. Selected extraction conditions of polyphenol were ethanol concentration 50%, ultrasonic power 500 W,

Table 3 Relative content of part of differential isoflavones metabolites in AMSE and FAMSE

Compounds	Relative content		VIP	FC	Type
	AMSE	FAMSE			
Daidzein	2.41E+05	2.55E+07	1.16	105.46	Up
Genistein	5.01E+05	1.47E+07	1.16	29.38	Up
Formononetin (7-Hydroxy-4′-methoxyisoflavone)	2.51E+07	5.09E+07	1.14	2.03	Up
Prunetin (5,4′-Dihydroxy-7-methoxyisoflavone)	1.14E+07	3.19E+07	1.16	2.80	Up
Pratensein	3.77E+06	1.12E+07	1.14	2.96	Up
4′,6,7-Trihydroxyisoflavone	5.04E+04	2.15E+06	1.16	42.69	Up
Isoluteolin (orobol)	4.68E+04	1.19E+06	1.16	25.4	Up
Glycitein	2.63E+06	6.30E+06	1.15	2.93	Up
5,7,4′-Trihydroxy-3′-methoxyisoflavone; 3′-*O*-methylorobol	1.38E+06	3.39E+06	1.15	2.45	Up
Iristectorigenin A	5.80E+05	1.82E+06	1.15	3.14	Up
3′-Methoxydaidzein	2.91E+05	9.97E+05	1.16	3.42	Up
2′-Hydroxydaidzein	1.03E+04	2.42E+05	1.16	23.52	Up
Biochanin A-7-*O*-glucoside-6″-*O*-malonate	3.62E+07	2.64E+06	1.14	0.07	Down
6″-*O*-Acetylglycitin	2.85E+07	2.76E+06	1.16	0.10	Down
Formononetin 7-*O*-(6″-acetylglucoside)	2.31E+07	2.68E+06	1.15	0.12	Down
Formononetin acetyl glucoside	1.93E+07	2.66E+06	1.16	0.14	Down
6″-*O*-Malonylgenistin	1.32E+07	1.64E+06	1.16	0.12	Down
Tectoridin	1.36E+07	1.43E+06	1.16	0.11	Down
Irilin B-7-*O*-malonyl glucoside	8.60E+06	1.36E+06	1.13	0.16	Down
Pratensein-7-*O*-glucoside	3.99E+06	1.31E+06	1.09	0.33	Down
Genistein-7-*O*-galactoside	3.92E+06	6.69E+05	1.15	0.17	Down
Prunetin-5-*O*-glucoside	4.37E+06	5.35E+05	1.16	0.12	Down
3′-Methoxydaidzin	4.19E+06	5.20E+05	1.15	0.12	Down
8-Methoxy malonyl ononin	7.57E+06	7.76E+05	1.16	0.10	Down
Afrormosin-7-*O*-(6″-malonyl)glucoside	4.78E+06	2.72E+05	1.15	0.06	Down

Table 4 Relative content of part of flavonols differential metabolites in AMSE and FAMSE

Compounds	Relative content		VIP	FC	Type
	AMSE	FAMSE			
3,5,4′-Trihydroxy-7-methoxyflavone (Rhamnocitrin)	3.71E+06	9.25E+06	1.15	2.49	Up
Galangin (3,5,7-Trihydroxyflavone)	7.14E+04	1.42E+06	1.16	19.9	Up
Flavoyadorinin A	1.32E+05	2.66E+05	1.01	2.01	Up
Rhamnetin	1.82E+04	8.86E+04	1.16	4.86	Up
Isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside	1.91E+04	4.17E+04	1.03	2.18	Up
Kaempferol-3-*O*-(6″-malonyl)galactoside	1.97E+07	1.52E+06	1.14	0.08	Down
Kaempferol-3-*O*-(6″-malonyl)glucoside	1.84E+07	1.46E+06	1.15	0.08	Down
6-C-MethylKaempferol-3-glucoside	1.51E+07	1.37E+06	1.16	0.09	Down
Kaempferol-3-*O*-(6″-*O*-acetyl)glucoside	2.34E+06	1.09E+05	1.15	0.05	Down
Kaempferol-3-*O*-(2″-*O*-acetyl)glucoside	1.97E+06	1.70E+05	1.15	0.09	Down
Kaempferide-3-*O*-glucoside	1.36E+06	5.33E+05	1.15	0.39	Down
Tamarixetin-3-*O*-(6″-malonyl)glucoside	1.05E+06	9.39E+04	1.13	0.09	Down
Tamarixetin-3-*O*-(6″-malonyl)glucoside	1.03E+06	4.72E+04	1.15	0.05	Down
6-C-MethylKaempferol-3-glucoside	5.41E+06	2.23E+04	1.16	0	Down
Isorhamnetin-7-*O*-glucoside (brassicin)	8.95E+05	4.97E+04	1.1	0.06	Down
Tamarixetin-3-*O*-(6″-malonyl)glucoside	9.52E+05	4.38E+04	1.15	0.05	Down
Kaempferide-3-*O*-(6′-*O*-acetyl)glucoside	9.42E+05	5.65E+04	1.15	0.06	Down
6-Hydroxykaempferol-3-*O*-Rutinoside-6-*O*-glucoside	7.07E+05	4.94E+04	1.16	0.07	Down

extraction temperature 35 °C, and extraction time 40 min. With these above conditions, the polyphenol and flavonoid contents as well as antioxidant activity of AM stems were increased. The relative contents of phenolic acids and flavonoid metabolites, DHFA, IFA, FA, apigenin, tricin, daidzein, et al., were significantly upregulated, which may be the reason for enhanced antioxidant activity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00674-x.

Additional file 1: Fig. S1. Phenolic acids metabolites identified in AMSE and FAMSE. (**A**) The three-dimensional scatter plot of the PCA model. (**B**) The verification chart of the OPLS-DA model. (C) Volcano plot of the differential phenolic acids metabolites. **Fig. S2.** Flavonoids metabolites identified in AMSE and FAMSE. (**A**) The two-dimensional scatter plot of the PCA model. (**B**) Permutation test of the OPLS-DA model. (**C**) Volcano plot of the differential flavonoids metabolites.

Acknowledgements
Not applicable.

Author contributions
N.L. methodology, investigation, formal analysis, writing—original draft, and writing—review and editing. XP. A. conceptualization, methodology, data curation and project funding acquisition. Y. W. methodology, validation and formal analysis. JW. Q. supervision, project administration and funding acquisition. Y. J. methodology and formal analysis. X. L. methodology. ZLG. Z. methodology. XN. C. methodology. All authors reviewed the manuscript.

Funding
This work was financially supported by the Outstanding Youth Science Foundation of Inner Mongolia Agricultural University (Grant no. BR230403), Major Science and Technology Program of Inner Mongolia Autonomous Region (Grant no. 2021ZD0023-3), National Center of Technology Innovation for Dairy Program (Grant no. 2022-scientific research-2, Grant no. 2023-QNRC-10) and Inner Mongolia Education Department Special Research Project for First Class Disciplines (grant number YLXKZX-NND-007).

Availability of data and materials
No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China. [2]National Center of Technology Innovation for Dairy-Breeding and Production Research Subcenter, Hohhot 010018, China. [3]Key Laboratory of Smart Animal Husbandry at Universities of Inner Mongolia Autonomous Region, Integrated Research Platform of Smart Animal Husbandry at Universities of Inner Mongolia, Inner Mongolia Herbivorous Livestock Feed Engineering Technology Research Center, Hohhot 010018, China.

Received: 22 May 2024 Accepted: 25 September 2024
Published online: 08 October 2024

References

1. Abdallah A, Zhang P, Zhong Q, Sun Z. Application of traditional Chinese herbal medicine by-products as dietary feed supplements and antibiotic replacements in animal production. Curr Drug Metab. 2019;20(1):54–64.
2. Adham AA, Sabry MB, Gamal AAR, Sabry AS. Effect of cinnamaldehyde thymol mixture on growth performance and some ruminal and blood constituents in growing lambs fed high concentrate diet. Life Sci J. 2014;11(3):240–8.
3. Kyuchukova R. Antibiotic residues and human health hazard-review. Bulg J Agric Sci. 2020;26(3):664–8.
4. Wang XJ, Ding LM, Wei HY, Jiang CX, Yan Q, Hu CS, et al. *Astragalus membranaceus* root supplementation improves average daily gain, rumen fermentation, serum immunity and antioxidant indices of Tibetan sheep. Animal. 2021;15(1): 100061.
5. Wu S. Effect of dietary Astragalus membranaceus polysaccharide on the growth performance and immunity of juvenile broilers. Poultry Sci. 2018;97(10):3489–93.
6. Wang R, Lin F, Ye C, Aihemaitijiang S, Halimulati M, Huang X, et al. Multi-omics analysis reveals therapeutic effects of *Bacillus subtilis*-fermented *Astragalus membranaceus* in hyperuricemia via modulation of gut microbiota. Food Chem. 2023;399: 133993.
7. Durazzo A, Nazhand A, Lucarini M, Silva AM, Souto SB, Guerra F, et al. *Astragalus* (*astragalus membranaceus bunge*): botanical, geographical, and historical aspects to pharmaceutical components and beneficial role. Rend Lincei Sci Fis. 2021;32(3):625–42.
8. Abdel-Latif HM, Ahmed HA, Shukry M, Chaklader MR, Saleh RM, Khallaf MA. *Astragalus membranaceus* extract (AME) enhances growth, digestive enzymes, antioxidant capacity, and immunity of *Pangasianodon hypophthalmus* juveniles. Fishes. 2022;7(6):319.
9. Ma L, Luo Z, Chen J, Du Z, Zhou T, Huang Y, et al. Effect of *Astragalus membranaceus* root on the serum metabolome of preweaning dairy calves. Agriculture. 2022;12(6):744.
10. Yao T, Zhang T, Zhao Q, Wang S, Chen Y, Ning Z, et al. Effects of dietary supplementation with astragalus (*Astragalus membranaceus*) root or leaf meal on the hematology, serum biochemical parameters, histomorphology, oxidative status, and resistance of Phoxinus lagowskii against bacterial infection. Aquaculture. 2023;565: 739135.
11. Yu J, Dong XD, Jiao JS, Ji HY, Liu AJ. Antitumor and immunoregulatory activities of a novel polysaccharide from *Astragalus membranaceus* on S180 tumor-bearing mice. Int J Biol Macromol. 2021;189:930–8.
12. D'Avino D, Cerqua I, Ullah H, Spinelli M, Di Matteo R, Granato E, et al. Beneficial effects of *Astragalus membranaceus* (fisch.) bunge extract in controlling inflammatory response and preventing asthma features. Int J Mol Sci. 2023;24(13):10954.
13. Jiao Y, Wen J, Yu X. Influence of flavonoid of *Astragalus membranaceus*'s stem and leaves on the function of cell mediated immunity in mice. Chin J Integr Tradit West Med. 1999;19(6):356–8.
14. Lekmine S, Boussekine S, Kadi K, Martín-García AI, Kheddouma A, Nagaz K, Bensouici C. A comparative study on chemical profile and biological activities of aerial parts (stems, flowers, leaves, pods and seeds) of *Astragalus gombiformis*. Biocatal Agric Biotechnol. 2020;27: 101668.
15. Khaled HB, Nayeem N. Physicochemical characteristics, anti-lipase and antioxidant activities of polysaccharide extracted from astragalus spinosis grown in the northern region of Saudi Arabia. J Pharm Res Int. 2021;33(43A):514–26.
16. Cui L, Ma Z, Wang D, Niu Y. Ultrasound-assisted extraction, optimization, isolation, and antioxidant activity analysis of flavonoids from *Astragalus membranaceus* stems and leaves. Ultraso Sonochem. 2022;90: 106190.
17. Nayeem N, Imran M, Asdaq SMB, Rabbani SI, Alanazi FA, Alamri AS, et al. Total phenolic, flavonoid contents, and biological activities of stem extracts of *Astragalus spinosus* (Forssk.) Muschl. grown in Northern Border Province, Saudi Arabia. Saudi J Biol Sci. 2022;29(3):1277–82.
18. Bei Q, Wu Z, Chen G. Dynamic changes in the phenolic composition and antioxidant activity of oats during simultaneous hydrolysis and fermentation. Food Chem. 2020;305: 125269.

19. Gonçalves S, Moreira E, Grosso C, Andrade PB, Valentão P, Romano A. Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. J Food Sci Tech. 2017;54:219–27.
20. Santini A, Tenore GC, Novellino E. Nutraceuticals: a paradigm of proactive medicine. Eur J Pharm Sci. 2017;96:53–61.
21. Zhou J, Wang M, Berrada H, Zhu Z, Grimi N, Barba FJ. Pulsed electric fields (PEF), pressurized liquid extraction (PLE) and combined PEF+ PLE process evaluation: Effects on Spirulina microstructure, biomolecules recovery and Triple TOF-LC-MS-MS polyphenol composition. Innov Food Sci Emerg. 2022;77: 102989.
22. Liu N, Wang Y, An X, Qi J, Wang B, Du J, Wang W. Composition, antioxidant and development-promoting activity of fermentation modified crude polysaccharides from stem and leaves of *Chenopodium album* L. Chem Biol Technol Agric. 2024;11(1):88.
23. Zhang L, Li X, Song X, Bian C, Kang X, Zhao J, et al. Assessment of the endophytic fungal composition of *Lactobacillus plantarum* and *Enterococcus faecalis*-fermented *Astragalus membranaceus* using single-molecule, real-time sequencing technology. Front Vet Sci. 2022;9: 880152.
24. Verni M, Rizzello CG, Coda R. Fermentation biotechnology applied to cereal industry by-products: nutritional and functional insights. Front Nutr. 2019;6:42.
25. Wang Y, Sun H, Han B, Li HY, Liu XL. Improvement of nutritional value, molecular weight patterns (soluble peptides), free amino acid patterns, total phenolics and antioxidant activity of fermented extrusion pretreatment rapeseed meal with Bacillus subtilis YY-1 and *Saccharomyces cerevisiae* Y. LWT. 2022;160: 113280.
26. Qiao H, Zhang X, Shi H, Song Y, Bian C, Guo A. Assessment of the physicochemical properties and bacterial composition of *Lactobacillus* plantarum and *Enterococcus faecium*-fermented *Astragalus membranaceus* using single molecule, real-time sequencing technology. Sci Rep. 2018;8(1):11862.
27. Li L, Wang L, Fan W, Jiang Y, Zhang C, Li J, et al. The application of fermentation technology in traditional Chinese medicine: a review. Am J Chin Med. 2020;48(04):899–921.
28. Bei Q, Chen G, Liu Y, Zhang Y, Wu Z. Improving phenolic compositions and bioactivity of oats by enzymatic hydrolysis and microbial fermentation. J Funct Foods. 2018;47:512–20.
29. Liu L, Zhang R, Deng Y, Zhang Y, Xiao J, Huang F, et al. Fermentation and complex enzyme hydrolysis enhance total phenolics and antioxidant activity of aqueous solution from rice bran pretreated by steaming with α-amylase. Food Chem. 2017;221:636–43.
30. Liu N, Wang Y, An X, Qi J. Study on the enhancement of antioxidant properties of rice bran using mixed-bacteria solid-state fermentation. Fermentation. 2022;8:212.
31. Liu N, Song M, Wang N, Wang Y, Qi J. The effects of solid-state fermentation on the content, composition and in vitro antioxidant activity of flavonoids from dandelion. PLoS ONE. 2020;15: e0239076.
32. Yin N, Wang Y, Ren X, Zhao Y, Liu N, An X, Qi J. Isolation and characterization of flavonoids from fermented dandelion (*Taraxacum mongolicum* Hand.-Mazz.), and assessment of its antioxidant actions in vitro and in vivo. Fermentation. 2022;8(7):306.
33. Liu N, An X, Wang Y, Qi J. Metabolomics analysis reveals the effect of fermentation to secondary metabolites of *Chenopodium album* L. based on UHPLC-QQQ-MS. Fermentation. 2023;9(2):100.
34. Park HM, Lee JY, Kim MY, Kang CH, Hwang HS. Anti-oxidative and anti-inflammatory activities of *Astragalus membranaceus* fermented by *Lactiplantibacillus plantarum* on LPS-induced RAW 264.7 cells. Fermentation. 2021;7(4):252.
35. Wang L, Luo Y, Wu Y, Liu Y, Wu Z. Fermentation and complex enzyme hydrolysis for improving the total soluble phenolic contents, flavonoid aglycones contents and bio-activities of guava leaves tea. Food Chem. 2018;264:189–98.
36. Gligor O, Mocan A, Moldovan C, Locatelli M, Crişan G, Ferreira IC. Enzyme-assisted extractions of polyphenols-a comprehensive review. Trends Food Sci Tech. 2019;88:302–15.
37. Siddiq M, Dolan KD, Perkins-Veazie P, Collins JK. Effect of pectinolytic and cellulytic enzymes on the physical, chemical, and antioxidant properties of blueberry (*Vaccinium corymbosum* L.) juice. Lwt. 2018;92:127–32.
38. Shen L, Pang S, Zhong M, Sun Y, Qayum A, Liu Y, et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: principles, advantages, equipment, and combined technologies. Ultrason Sonochem. 2023;13: 106646.
39. Xie Z, Sun Y, Lam S, Zhao M, Liang Z, Yu X, et al. Extraction and isolation of flavonoid glycosides from *Flos Sophorae Immaturus* using ultrasonic-assisted extraction followed by high-speed countercurrent chromatography. J Sep Sci. 2014;37(8):957–65.
40. Agarwal C, Máthé K, Hofmann T, Csóka L. Ultrasound-assisted extraction of cannabinoids from *Cannabis sativa* L. optimized by response surface methodology. J Food Sci. 2018;83(3):700–10.
41. Iftikhar M, Zhang H, Iftikhar A, Raza A, Begum N, Tahamina A, et al. Study on optimization of ultrasonic assisted extraction of phenolic compounds from rye bran. Lwt. 2020;134: 110243.
42. Ma YQ, Ye XQ, Fang ZX, Chen JC, Xu GH, Liu DH. Phenolic compounds and antioxidant activity of extracts from ultrasonic treatment of satsuma mandarin (*Citrus unshiu* Marc.) peels. J Agric Food Chem. 2008;56:5682–90.
43. Liu JL, Li LY, He GH. Optimization of microwave-assisted extraction conditions for five major bioactive compounds from Flos Sophorae immaturus (cultivars of *Sophora japonica* L.) using response surface methodology. Molecules. 2016;21(3):296.
44. Zhang J, Li M, Cheng J, Zhang X, Li K, Li B, et al. *Viscozyme* L. hydrolysis and *Lactobacillus* fermentation increase the phenolic compound content and antioxidant properties of aqueous solutions of quinoa pretreated by steaming with α-amylase. J Food Sci. 2021;86(5):1726–36.
45. Dong W, Fan Z, Li P, Liu J, Sun G, Peng N, et al. Optimizing the scale-up production of fermented astragalus and its benefits to the performance and egg quality of laying hens. Front Microbiol. 2023;14:1165644.
46. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Bio Med. 2010;49(11):1603–16.
47. Turkan I. ROS and RNS: key signalling molecules in plants. J Exp Bot. 2018;69(14):3313–5.
48. Kuo HC, Kwong HK, Chen HY, Hsu HY, Yu SH, Hsieh CW, et al. Enhanced antioxidant activity of *Chenopodium formosanum* Koidz. by lactic acid bacteria: optimization of fermentation conditions. PLoS ONE. 2021;16(5): e0249250.
49. Zhang H, Tsao R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opin Food Sci. 2016;8:33–42.
50. Magani SK, Mupparthi SD, Gollapalli BP, Shukla D, Tiwari AK, Gorantala J, et al. Salidroside-can it be a multifunctional drug? Curr Drug Metab. 2020;21(7):512–24.
51. Wu M, Luo Q, Nie R, Yang X, Tang Z, Chen H. Potential implications of polyphenols on aging considering oxidative stress, inflammation, autophagy, and gut microbiota. Crit Rev Food Sci Nutr. 2021;61(13):2175–93.
52. Boudaoud S, Aouf C, Devillers H, Sicard D, Segond D. Sourdough yeast-bacteria interactions can change ferulic acid metabolism during fermentation. Food Microbiol. 2021;98: 103790.
53. Serreli G, Naitza MR, Zodio S, Leoni VP, Spada M, Melis MP, et al. Ferulic acid metabolites attenuate LPS-induced inflammatory response in enterocyte-like cells. Nutrients. 2021;13(9):3152.
54. Amić A, Marković Z, Klein E, Marković JMD, Milenković D. Theoretical study of the thermodynamics of the mechanisms underlying antiradical activity of cinnamic acid derivatives. Food chem. 2018;246:481–9.
55. Ohue-Kitano R, Taira S, Watanabe K, Masujima Y, Kuboshima T, Miyamoto J, et al. 3-(4-Hydroxy-3-methoxyphenyl) propionic acid produced from 4-Hydroxy-3-methoxycinnamic acid by gut microbiota improves host metabolic condition in diet-induced obese mice. Nutrients. 2019;11(5):1036.
56. Lee SH, Yeo D, Hong JH. Effect of dihydroferulic acid obtained from fermented rice bran extract on neuroprotection and behavioral recovery in an ischemic rat model. Food Sci Tech. 2020;40:475–81.
57. Long Z, Feng G, Zhao N, Wu L, Zhu H. Isoferulic acid inhibits human leukemia cell growth through induction of G2/M-phase arrest and inhibition of Akt/mTOR signaling. Mol Med Rep. 2020;21(3):1035–42.
58. Masuda T, Yamada K, Maekawa T, Takeda Y, Yamaguchi H. Antioxidant mechanism studies on ferulic acid: isolation and structure identification of the main antioxidation product from methyl ferulate. Food Sci Technol Res. 2006;12(3):173–7.

59. Qiu X, Jacobsen C, Villeneuve P, Durand E, SoRensen ADM. Effects of different lipophilized ferulate esters in fish oil-enriched milk: partitioning, interaction, protein, and lipid oxidation. J Agric Food Chem. 2017;65(43):9496–505.
60. Chen C. Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. Oxid Med Cell Longev. 2016;2016:3571614.
61. Velika B, Kron I. Antioxidant properties of benzoic acid derivatives against superoxide radical. Free Radic Antioxid. 2012;2(4):62–7.
62. He L, Li S, Wang C, Chen X, Zhang Q. Effects of vanillic acid on dynamic fermentation parameter, nitrogen distribution, bacterial community, and enzymatic hydrolysis of stylo silage. Front Microbiol. 2021;12: 690801.
63. Vo QV, Bay MV, Nam PC, Quang DT, Flavel M, Hoa NT, Mechler A. Theoretical and experimental studies of the antioxidant and antinitrosant activity of syringic acid. J Org Chem. 2020;85(23):15514–20.
64. Abedi F, Razavi BM, Hosseinzadeh H. A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: comprehensive pharmacology, toxicology, and some pharmaceutical aspects. Phytother Res. 2020;34(4):729–41.
65. Degrain A, Manhivi V, Remize F, Garcia C, Sivakumar D. Effect of lactic acid fermentation on color, phenolic compounds and antioxidant activity in African nightshade. Microorganisms. 2020;8(9):1324.
66. Darwesh OM, Eweys AS, Zhao YS, Matter IA. Application of environmental-safe fermentation with *Saccharomyces cerevisiae* for increasing the cinnamon biological activities. BIOB. 2023;10(1):12.
67. Azkia MN, Cahyanto MN, Mayangsari Y, Briliantama A, Palma M, Setyaningsih W. Enhancement of phenolic profile and antioxidant activity of black glutinous rice (*Oryza sativa* var. glutinosa) due to tape fermentation. Arab J Chem. 2023;16(11): 105275.
68. Yoo JA, Lim YM, Yoon MH. Production and antifungal effect of 3-phenyllactic acid (PLA) by lactic acid bacteria. J Appl Biol Chem. 2016;59(3):173–8.
69. Zhang YY, Li XL, Li TY, Li MY, Huang RM, Li W, Yang RL. 3-(4-Hydroxyphenyl) propionic acid, a major microbial metabolite of procyanidin A2, shows similar suppression of macrophage foam cell formation as its parent molecule. RSC Adv. 2018;8(12):6242–50.
70. Zhang B, Zhang Y, Liu X, Zhao C, Yin J, Li X, et al. Distinctive anti-inflammatory effects of resveratrol, dihydroresveratrol, and 3-(4-hydroxyphenyl)-propionic acid on DSS-induced colitis in pseudo-germ-free mice. Food Chem. 2023;400: 133904.
71. Karak P. Biological activities of flavonoids: an overview. Int J Pharm Sci Res. 2019;10(4):1567–74.
72. Kashyap P, Shikha D, Thakur M, Aneja A. Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: a review. J Food Biochem. 2022;46(4): e13950.
73. Li M, Pu Y, Yoo CG, Ragauskas AJ. The occurrence of tricin and its derivatives in plants. Green Chem. 2016;18(6):1439–54.
74. Huwait E, Mobashir M. Potential and therapeutic roles of diosmin in human diseases. Biomedicines. 2022;10(5):1076.
75. Singh S, Gupta P, Meena A, Luqman S. Acacetin, a flavone with diverse therapeutic potential in cancer, inflammation, infections and other metabolic disorders. Food Chem Toxicol. 2020;145: 111708.
76. Barna D, Alshaal T, Tóth IO, Cziáky Z, Fári MG, Domokos-Szabolcsy É, Bákonyi N. Bioactive metabolite profile and antioxidant properties of brown juice, a processed alfalfa (*Medicago sativa*) by-product. Heliyon. 2022;8(11): e11655.
77. Chen L, Teng H, Xie Z, Cao H, Cheang WS, Skalicka-Woniak K, et al. Modifications of dietary flavonoids towards improved bioactivity: an update on structure-activity relationship. Crit Rev Food Sci Nutr. 2018;58(4):513–27.
78. Sheng Z, Jiang Y, Liu J, Yang B. UHPLC–MS/MS analysis on flavonoids composition in *Astragalus membranaceus* and their antioxidant activity. Antioxidants. 2021;10(11):1852.
79. Chiorcea-Paquim AM, Enache TA, De Souza GE, Oliveira-Brett AM. Natural phenolic antioxidants electrochemistry: towards a new food science methodology. Compr Rev Food Sci F. 2020;19(4):1680–726.
80. Yu L, Zhang Y, Chen Q, He Y, Zhou H, Wan H, Yang J. Formononetin protects against inflammation associated with cerebral ischemia-reperfusion injury in rats by targeting the JAK2/STAT3 signaling pathway. Biomed Pharmacother. 2022;149: 112836.
81. Hu H, Li H. Prunetin inhibits lipopolysaccharide-induced inflammatory cytokine production and MUC5AC expression by inactivating the TLR4/MyD88 pathway in human nasal epithelial cells. Biomed Pharmacother. 2018;106:1469–77.
82. Yang G, Ham I, Choi HY. Anti-inflammatory effect of prunetin via the suppression of NF-κB pathway. Food Chem Toxicol. 2013;58:124–32.
83. Liu CH, Zhang WD, Wang JJ, Feng SD. Senegenin ameliorate acute lung injury through reduction of oxidative stress and inhibition of infammation in cecal ligation and puncture-induced sepsis rats. Infammation. 2016;39(2):900–90.
84. Lee JY, Park HM, Kang CH. Antioxidant effect via bioconversion of isoflavonoid in *Astragalus membranaceus* fermented by *Lactiplantibacillus plantarum* MG5276 in vitro and in vivo. Fermentation. 2022;8(1):34.
85. Baek SC, Park MH, Ryu HW, Lee JP, Kang MG, Park D, et al. Rhamnocitrin isolated from *Prunus padus* var. seoulensis: a potent and selective reversible inhibitor of human monoamine oxidase A. Bioorg Chem. 2019;83:317–25.
86. Singh D, Saini A, Singh R, Agrawal R. Galangin, as a potential anticancer agent. Rev Bras Farmacogn. 2022;32(3):331–43.
87. Shakour ZTA, Fayek NM, Farag MA. How do biocatalysis and biotransformation affect Citrus dietary flavonoids chemistry and bioactivity? A review Crit Rev Biotechnol. 2020;40(5):689–714.
88. Lee NK, Paik HD. Bioconversion using lactic acid bacteria: ginsenosides, GABA, and phenolic compounds. J Microbiol Biotechn. 2017;27(5):869–77.
89. Ravisankar S, Queiroz VA, Awika JM. Rye flavonoids-structural profile of the flavones in diverse varieties and effect of fermentation and heat on their structure and antioxidant properties. Food Chem. 2020;324: 126871.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.