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Characterization of exopolysaccharides and metabolic profile in goldenberry (*Physalis peruviana* L.) fermented by *Leuconostoc mesenteroides*

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ABSTRACT: Goldenberry (*Physalis peruviana* L.) is a nutrient-rich fruit containing abundant flavonoids and other bioactive compounds with health-promoting properties. For enhancing its functional potential, goldenberry was fermented by *Leuconostoc mesenteroides* DRP105 to produce exopolysaccharides (EPS) and investigate related metabolic shifts. The isolated EPS was identified as a dextran (6.49×10^5 Da) with α -(1→6)-linked glucose units. Metabolomic profiling during fermentation identified significant alterations, with 8 compounds upregulated and 31 downregulated. Notably, a substantial decline was observed in most native flavonoids and their derivatives, especially 3,5,7,3',4'-pentahydroxyflavanone. Flavonoids undergo biotransformation into derivatives with enhanced stability and bioavailability. Concurrently, EPS and taxifolin form a noncovalent complex that enhance antioxidant activity. This work provides valuable insights for designing novel functional foods functionally enhanced with these compounds and establishes a theoretical basis for the expanded utilization of EPS synthesized from fruit substrates.

Keywords: Exopolysaccharides, Goldenberry, *Leuconostoc mesenteroides*, Metabolite, Fermentation process

1. Introduction

Goldenberry (*Physalis peruviana* L.) is an upright, semi-shrubby perennial native to tropical and subtropical regions, producing orange-yellow berries 1.25-2 cm in diameter. It is native to the Andean region, particularly Ecuador and Peru^[1]. The nutrients of goldenberry contain protein, ash, fat, dietary fiber, carbohydrates, and are abundant in vitamin C, β -carotene, total phenolic compounds, flavonoids, and withanolide^[2]. Additionally, it exhibits multiple bioactivities, including antispasmodic, diuretic, and antidiabetic properties. Furthermore, it purifies kidney blood, reduces albumin levels, clears cataracts, and lowers blood glucose, and is widely used in folk medicine^[3]. Goldenberry can also be processed into jams, raisins chocolate-covered candies, and juice.

Nutritious and functional beverages with high added value have been attracting growing attention. Fermentation, as a traditional method in food processing, is extensively employed to prolong the shelf life of

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raw ingredients and improve their sensory attributes^[4, 5]. Lactic acid bacteria (LAB) are generally recognized as safe (GRAS). Additionally, LAB fermentation can improve the flavor profile and boost the antioxidant activity of products^[6]. Fermentation of goji juice with *Lactocaseibacillus rhamnosus* significantly reduced soluble protein, total phenolic compounds, flavonoids, and sugar content while producing novel volatile compounds and enhancing antioxidant capacity^[4]. Fermented *Opuntia ficus-indica* fruit juice by LAB altered the acid and sugar contents, increased the total phenolic and anthocyanin contents, and enhanced the antioxidant activity^[7]. In the fermentation process, important components of fruit juice can be altered, endowing it with functional activity^[8]. LAB fermentation has been considered a valuable biotechnological method, which maintains and improves the nutritional, sensory, safety, and shelf-life of fruits^[9].

Exopolysaccharides (EPS) are polysaccharide polymers secreted by LAB. They consist of branched, repeating units of sugars or sugar derivatives in varying ratios, with primary sugar components including glucose, galactose, and rhamnose^[10]. EPS are generally classified as homopolysaccharides (HoPS), consisting of only one type of monosaccharide, or heteropolysaccharides (HePS), composed of two or more distinct monosaccharides. Owing to their unique physicochemical and rheological properties, EPS exhibit diverse applications in the food industry. Furthermore, EPS possess excellent bioactivities, such as cholesterol-lowering, immunomodulatory, antioxidant, antiviral, and anticoagulant effects^[11]. Recent investigations have emphasized the development, biological activity, and practical implementations of complexes formed between polysaccharides and polyphenolic compounds (PPCs), indicating their roles in gut microbiota modulation, antioxidant delivery, and anti-inflammatory effect^[12, 13]. For instance, pre-fermentation of litchi pulp with *Lactobacillus fermentum* produced polysaccharides with lower molecular weight and enhanced proliferative activity, while improving its fermentation properties and prebiotic potential^[14]. In another study, LAB fermentation of mango juice improved its antioxidant activity; metabolomic analysis revealed that polyphenols were the primary contributors to this increased antioxidant capacity. Moreover, fermentation of *Rosa roxburghii* Tratt juice notably increased the contents of total phenolics, flavonoids, and certain bioactive compounds, while notably enhancing its antioxidant capacity and α -glucosidase inhibitory activity. Conversely, its cholesterol esterase inhibitory activity was significantly reduced^[15].

This study focused on EPS production from goldenberry by liquid fermentation with *Leuconostoc mesenteroides* DRP105, as well as the subsequent purification and structural–functional characterization of the EPS. Specifically, goldenberry was selected as the fermentation substrate because it provides essential nutrients to support the proliferation of *L. mesenteroides* and the biosynthesis of EPS. Moreover, the fruit is naturally rich in flavonoids and polyphenols. During fermentation, these phytochemicals undergo biotransformation, potentially enhancing their bioactivity. Concurrently, metabolomic analyses of the fermenting juice at two distinct time points (0 h and 60 h) were performed to identify key metabolic shifts. This study focuses on characterizing the structure and bioactivity of EPS while exploring related metabolic

alterations, and further investigating the interactions and synergistic effects between flavonoids and EPS to provide a theoretical basis for the application of fermentation products.

2. Materials and methods

2.1. Revival and cultivation of bacterial strain

L. mesenteroides DRP105, originally isolated from sauerkraut^[16], was revived and cultured in MRS broth at 30 °C , 120 rpm for 36 h.

2.2. Establishment of goldenberry juice fermentation and process analysis

Fresh goldenberries were washed, crushed, and filtered through four layers of sterile gauze to remove seeds and pulp. The resulting juice was diluted with distilled water at a ratio of 1:1 (v/v) and pasteurized at 80 °C for 15 min. The juice was inoculated with 2% (v/v) seed culture (1×10^6 CFU/mL) and fermented (25 °C , 96 h). Fermentation broth was collected every 12 h for pH measurement, viable cell counts, and EPS quantification by the phenol-sulfuric acid method^[17].

2.3. Extraction and purification of EPS

EPS extraction was performed according to the method described by Du et al.^[18]. Post fermentation broth was centrifuged, and the supernatant was precipitated with three volumes of chilled 95% ethanol (12 h, 4 °C). The precipitate was collected by centrifugation, dissolved in water, and treated with 80% (w/v) trichloroacetic acid overnight at 4 °C to remove proteins, followed by ethanol reprecipitation. Following 48 h dialysis at 4 °C with dialysis bags (flat width 77 mm; MWCO 8,000–14,000 Da), EPS purification employed Sephadex G-100 chromatography (1.6 × 50 cm, water, 2 mL/min, detection at 220 nm), lyophilized (SJIA-10 N-80C, -80 °C , 12 h), and analyzed by UV-Vis spectroscopy (190–400 nm, UV-2550, Shimadzu, Japan). The purified, lyophilized EPS powder was stored in sealed centrifuge tubes at -20 °C to prevent moisture absorption and degradation.

2.4. Characterization of chemical composition and structural properties

2.4.1. Elemental and biochemical composition

The elemental composition of EPS was analyzed with a Vario MACRO cube elemental analyzer (Elementar, Germany). Total carbohydrate and protein contents were measured by the phenol-sulfuric acid method^[19] and the Bradford method^[20], respectively.

2.4.2. Molecular weight (*M_w*) determination

M_w of EPS was determined by HPSEC on an LC-10Avp system (Shimadzu, Kyoto, Japan) equipped with an RID-10A detector and a TSK-gel G-3000PWXL column (7.8 × 300 mm) at 40 °C . Samples (2 mg, 5 mg/mL in 0.2 M NaCl) were injected at 20 µL, with 0.2 M NaCl as the mobile phase at 0.6 mL/min.

2.4.3. Monosaccharide profiling

According to Du et al.^[21], EPS (1 mg) was hydrolyzed with 1 M HCl in anhydrous methanol at 80 °C for 16 h, dried, and further hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 1 h. The hydrolysate

was derivatized with 0.5 M PMP (in methanol) and 0.3 M NaOH at 70 °C for 30 min. After neutralization, the derivatives were extracted three times with dichloromethane, filtered (0.22 µm), and analyzed using a Shimadzu LC-20AT HPLC system.

2.4.4 Glycosidic linkages determination

The structural characteristics of EPS were determined by nuclear magnetic resonance (NMR) spectroscopy according to Liang^[22]. Lyophilized EPS (50 mg) was subjected to three deuterium exchange cycles with D₂O to remove residual protons, dissolved in 0.55 mL D₂O, and analyzed on a Bruker Avance III NMR spectrometer (Bruker BioSpin, Germany) using standard 5-mm tubes. One-dimensional (¹H and ¹³C) and two-dimensional (HSQC, HMBC and COSY) spectra were recorded at 298 K, with chemical shifts referenced to residual solvent signals and expressed in ppm. Spectral processing and analysis were performed using MestReNova v14.2.1 (Mestrelab Research, Spain).

2.4.5. Surface microstructure determination

SEM was employed to characterize the microstructural features of EPS. Samples were sputter-coated with gold before imaging, and observations were conducted at an accelerating voltage of 8 kV with micrographs captured at representative magnifications (×100 and ×1,000).

2.5. EPS properties evaluation

2.5.1. Thermal property determination

The thermal behavior of EPS was characterized by TGA, DTG, and DSC (Maia F3 200, Netzsch, Germany) according to the method of Wang et al.^[23]. Approximately 3 mg of sample was heated from 40 to 800 °C at 10 °C /min under nitrogen (50 mL/min) in an alumina crucible.

2.6. Untargeted metabolomic analysis during fermentation

2.6.1. Metabolomics by LC-MS/MS

Fermentation broth (100 µL) was extracted with 400 µL methanol–acetonitrile (1:1, v/v), vortexed, ultrasonicated (10 min, 4 °C), incubated (1 h, -40 °C), and centrifuged (12,000 × g, 15 min, 4 °C). Supernatants were measured on a Vanquish UHPLC system (Thermo Fisher) with a Waters ACQUITY BEH Amide column (2.1 × 50 mm, 1.7 µm). The mobile phase was (A) 25 mmol/L ammonium acetate–ammonia in water and (B) acetonitrile, with a 2 µL injection at 4 °C. MS/MS spectra were acquired on an Orbitrap Exploris 120 (Thermo) in IDA mode under the following settings: sheath gas 50 Arb, auxiliary gas 15 Arb, capillary 320 °C, full MS resolution 60,000, MS/MS resolution 15,000, SNCE 20/30/40, spray voltage +3.8/–3.4 kV. All metabolomic analyses were performed in triplicate.

2.6.2. Multivariate Data Processing

Statistical evaluation employed multivariate techniques on normalized and logarithmically transformed datasets. Principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) analyses were performed using the MetaboAnalyst platform.

2.7. Characterization of EPS–flavonoid interactions

100 mg of EPS was dissolved in 10 mL of deionized water, and 20 mg of taxifolin was dissolved in 5 mL of deionized water followed by the addition of 2 mL of anhydrous ethanol. The two solutions were mixed and stirred for 1 h, subsequently dialyzed for 24 h (flat width 77 mm; M_w 8,000–14,000 Da), and finally lyophilized prior to analysis.

2.7.1. FT-IR spectroscopy

Fourier-transform infrared spectra were obtained using a BIO-RAD FTS3000 spectrometer (Bruker, Karlsruhe, Germany). EPS, taxifolin and EPS-taxifolin mixture were prepared as KBr discs, and spectra were recorded from 400–4000 cm^{-1} by averaging 32 scans.

2.7.2. X-ray diffraction (XRD)

XRD was employed to determine the crystalline structure of EPS, taxifolin and EPS-taxifolin mixture. Diffraction patterns were recorded on a powder diffractometer (X'Pert PRO MPD, Panalytical, Almelo, The Netherlands) at a scan rate of $2^\circ/\text{min}$ across a 2θ range of $10\text{--}80^\circ$.

2.7.3. Isothermal titration calorimetry (ITC) measurements

The thermodynamic parameters of the interaction between taxifolin and EPS were determined using a Nano ITC (TA Instruments, USA). Briefly, the sample cell (300 μL) was loaded with 0.01 mM EPS, while the titration syringe (50 μL) was filled with 0.2 mM taxifolin. All measurements were carried out at 25°C with a constant stirring speed of 350 rpm to ensure rapid and thorough mixing. The titration protocol consisted of 20 successive injections of 2 μL each, with a 120 s interval between consecutive injections to allow the system to fully return to thermal equilibrium. To correct for background heat effects, a control experiment was performed by titrating taxifolin into the blank solvent under identical experimental conditions. The heat of dilution obtained from this control was subtracted from the experimental raw data prior to curve fitting and thermodynamic analysis.

2.7.4. Antioxidant activity of EPS-taxifolin interaction

The EPS-Taxifolin interaction systems were prepared by mixing taxifolin and EPS solutions at a ratio of 1:1 (v/v), followed by incubation at 37°C for 60 min. In the final reaction mixtures, the concentrations of EPS were set at 0.25, 0.5, 1.0, 2.0, and 5.0 mg/mL. The final concentrations of Taxifolin were fixed at 0.1 mg/mL (0.01%, w/v) for the DPPH assay and 0.06 mg/mL (0.006%, w/v) for the ABTS assay.

The radical scavenging capacities of EPS alone and the EPS-Taxifolin interaction systems were evaluated using DPPH and ABTS assays, following the methodology described by Amiri et al.^[24] Ascorbic acid served as the positive reference standard. For the ABTS assay: A 2 mL aliquot of the sample (EPS solution or EPS-Taxifolin mixture) was added to 4 mL of the ABTS working solution. The absorbance was measured at 734 nm after reacting for 6 min in the dark. For the DPPH assay: A 2 mL aliquot of the sample was mixed with the DPPH solution. The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm.

2.8. Statistical analysis

Statistical analysis involved triplicate experimental replicates, with data expressed as mean $\pm$ standard deviation. ANOVA and Tukey's test were performed with JMP Pro 17 (SAS Institute, Cary, NC, USA). Significance was set at $P < 0.05$.

3. Results and discussion

3.1. Variation of pH, colony number, and EPS content during fermentation system

As shown in Fig. 1, the pH exhibited a general decreasing trend, dropping from an initial value of 4.10 to 3.87. It declined rapidly up to 72 h, with the most pronounced decrease occurring between 12 and 24 h, followed by a slight increase from 3.82 to 3.87 toward the end of the process. The pH decrease was mainly due to the formation of acidic metabolites produced by *L. mesenteroides* DRP105 during goldenberry fermentation^[25]. The slight pH increase observed later in the fermentation may be due to a reduction in bacterial metabolic activity as the culture entered the decline phase.

The bacterial growth curve exhibited four distinct phases. The colony count reached a peak at 36 h (3.16 log CFU/mL), then decreased until 60 h, attaining a local minimum of 2.15 log CFU/mL. A subsequent increase occurred from 60 h to 84 h, followed by a final decline from 84 h to 96 h. In the initial fermentation stage, the broth-rich in nutrients derived from goldenberry, including carbohydrates (e.g., fructose, glucose, sucrose), proteins, and inorganic salts supported the rapid growth of *L. mesenteroides* DRP105, resulting in a peak cell density. As nutrients were depleted, the CFU began to decline. Compared to *Lactobacillus helveticus* MB2-1 cultured in whey powder, which typically reaches maximum CFU at 25 h^[26], *L. mesenteroides* DRP105 in goldenberry broth showed an earlier decline starting at 36 h, likely due to quicker nutrient exhaustion. A transient increase in CFU observed around 60 h may reflect the utilization of alternative carbon sources, such as goldenberry-derived polysaccharides and other energy-yielding compounds, enabling temporary metabolic recovery and renewed growth.

The EPS content initially reached a peak of 25.71 ± 0.20 mg/mL in the first stage (12–36 h), then decreased rapidly during the second stage (36–48 h), reaching a minimum of 20.26 ± 0.20 mg/mL. Subsequently, the EPS level showed repeated fluctuations before increasing again, attaining a second peak of 21.12 ± 0.03 mg/mL. The concentration then continued to rise, ultimately reaching 22.88 ± 0.08 mg/mL at 96 h. During the early fermentation stage, the abundant sucrose in the broth supported high EPS production, culminating in the maximum yield at 36 h. This value is higher than that reported by Yang et al.^[27], who obtained an EPS yield of 11.29 ± 0.25 g/L from sugarcane bagasse. The synthesis of EPS by *L. mesenteroides* DRP105 is facilitated by glucosyltransferase, which utilizes sucrose from goldenberry as a substrate. In the mid to late stages of fermentation, as sugar resources decline, EPS may be hydrolyzed and reused by bacterial cells, leading to the observed fluctuations in EPS content.

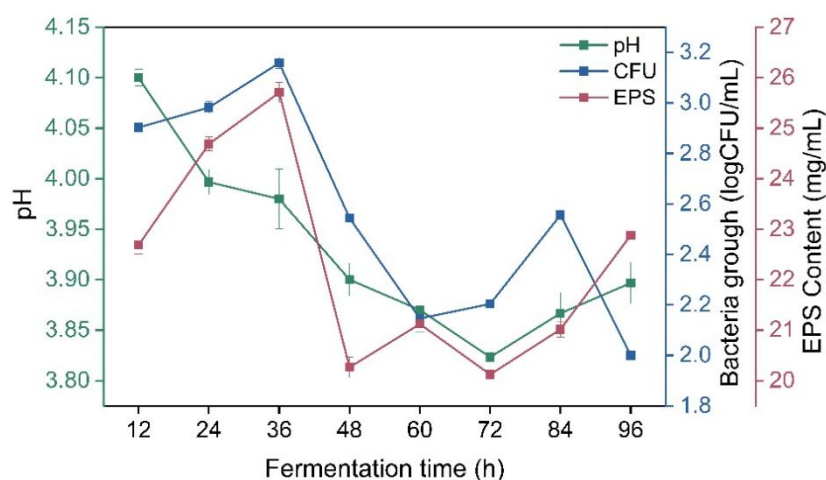


Fig. 1 Fermentation profile: pH, bacterial count, and EPS content.

3.2. EPS chemical composition analysis

Following the investigation of fermentation process and EPS yield, it is essential to isolate the EPS to verify its purity and elucidate its specific chemical composition. Pure EPS was obtained through a series of separation and purification steps, and its purity was assessed by UV-Vis spectroscopy (Fig. 2A) and elemental analysis. The UV spectrum exhibited a maximum absorption peak near 200 nm (characteristic of polysaccharide absorption) but showed no absorption at 260 nm or 280 nm, confirming the effective removal of nucleic acids and proteins. The elemental analysis further supported this high purity, showing that the EPS consisted of C (38.01%), H (6.44%), and negligible amounts of N (0.13%) and S (0%).

3.3. Mw distribution of EPS

The HPSEC chromatogram of the EPS displayed a single peak, confirming the purity. Based on a calibration curve generated with pullulan standards, the Mw of the EPS was determined to be 6.49×10^5 Da, indicating a homogeneous molecular size distribution. Mw is a critical factor influencing the EPS properties, with different sizes conferring distinct effects on viscosity, antioxidant capacity, and probiotic activity^[28]. Genetic variation among LAB affects the properties of EPS-synthesizing enzymes. For example, *Lactobacillus delbrueckii* subsp. *bulgaricus* generally produces high Mw EPS, while *Lactobacillus acidophilus* typically yields EPS with lower Mw^[29, 30]. High Mw EPS ($>10^6$ Da), known for strong water-holding and thickening capabilities, finds extensive applications in improving the texture of yogurt and cheese. In contrast, low Mw EPS ($<10^5$ Da) are more easily absorbed and are often preferred as functional ingredients in probiotic beverages^[31]. Notably, EPS with intermediate Mw (10^5 - 10^6 Da) frequently exhibit enhanced antioxidant and immunomodulatory activities, which may be attributed to their balanced ability to penetrate cell membranes while maintaining bioactive structural conformations^[32].

3.4. Compositional and structural properties of EPS

3.4.1. Monosaccharide composition

HPLC analysis revealed the monosaccharide composition of the EPS. As shown in Fig. 2B, the EPS consisted primarily of glucose, indicating that it is a glucan. EPS monosaccharide composition is a key factor

governing their structural diversity and functional specificity. The type, ratio, and glycosidic linkages of the constituent sugars directly affect the physicochemical characteristics and biological activities of EPS^[33]. For instance, the EPS produced by *Streptococcus thermophilus* S-3 composed of N-acetyl-galactosamine, galactose, and glucose in a 1:2:1 ratio imparts high viscosity and water-holding capacity to fermented milk, making it a potential partial substitute for commercial thickeners^[34]. In contrast, EPS from *Lactiplantibacillus plantarum* L3, which contains mannose and glucuronic acid, demonstrates significant immunomodulatory activity^[35].

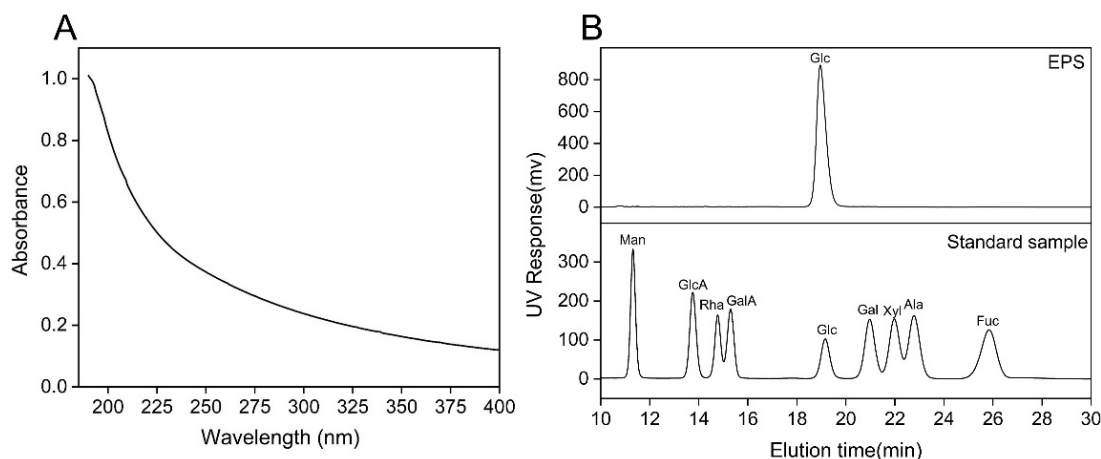


Fig. 2 Purity assessment and compositional analysis of EPS. (A) UV-Vis absorption spectrum, (B) monosaccharide composition.

3.4.2. Glycosidic linkage composition of EPS

NMR spectroscopy is a powerful tool for accurately and comprehensively elucidating the structural features of EPS, including the composition and proportions of sugar residues, and glycosidic bond configurations^[36]. In ^1H NMR spectrum (Fig. 3A), resonance signals were clearly observed in the anomeric proton region (δ 4.5–5.4 ppm), which is indicative of glycosidic linkage types, and the ring proton region (δ 3.2–4.4 ppm). A prominent anomeric proton signal at δ 4.92 ppm was observed, characteristic of α -(1 $\rightarrow$ 6) glycosidic linkages^[37]. Proton signals in δ 3.4–4.1 ppm were assigned to H2–H6 of the glucose residues. Based on the ^{13}C NMR spectrum (Fig. 3B), the anomeric carbon signal of α -configured residues appeared at δ 97.65 ppm, while no signals corresponding to β -anomeric carbons were detected, indicating the absence of β -configurations^[38]. Ring carbon signals (C-2–C-6) were found between δ 65–74 ppm, including δ 73.34 (C-3), 71.34 (C-2), 70.12 (C-5), 69.45 (C-4), and 65.46 ppm (C-6). These chemical shifts are consistent with glucosyl units substituted at the corresponding positions. Based on comparisons with previously reported data for α -(1 $\rightarrow$ 6)-linked dextran^[37], the signal at δ 65.46 ppm is diagnostic of 6-substituted glucopyranose residues^[22].

2D NMR (Fig. 3C–E) provided further confirmation of the structural details. Within the HSQC spectrum, direct ^1H – ^{13}C correlations were observed for the following pairs: δ 4.93/97.64 ppm (H-1/C-1), 3.67/71.34 ppm (H-2/C-2), 3.72/73.34 ppm (H-3/C-3), 3.47/69.45 ppm (H-4/C-4), 3.86/70.12 ppm (H-5/C-5), and 3.94, 3.71/65.46 ppm (H-6, H-6'/C-6), confirming the repeating α -(1 $\rightarrow$ 6)-linked glucosyl residues^[39]. HMBC analysis showed a long-range correlation of the anomeric proton (H-1, δ 4.93 ppm) and the downfield-shifted

C-6 signal (δ 65.46 ppm), directly confirming that the glycosidic linkage occurs at the C-6 position. This provides compelling evidence for an unbranched α -(1 $\rightarrow$ 6) glucan structure^[40]. In the COSY spectrum, scalar coupling correlations were observed between the following proton pairs: δ 4.93/3.67 ppm (H-1/H-2), 3.86/3.94 ppm (H-5/H-6), and 3.94/3.71 ppm (H-6/H-6'). The clear cross-peaks between H-5 and both H-6 protons, as well as the strong geminal correlation between H-6 and H-6', are characteristic of the α -(1 $\rightarrow$ 6)-linked glucopyranosyl residue. These findings are consistent with FT-IR data. The glycosidic linkage pattern of the EPS was similar to that previously reported in *Leuconostoc lactis* L2 and *L. mesenteroides* fermented in beet waste liquid^[39, 41]. Notably, linear EPS have been reported to exhibit superior solubility and viscosity, which enhance their functionality in food systems^[42]. Therefore, the EPS shows promising potential for applications in the food industry.

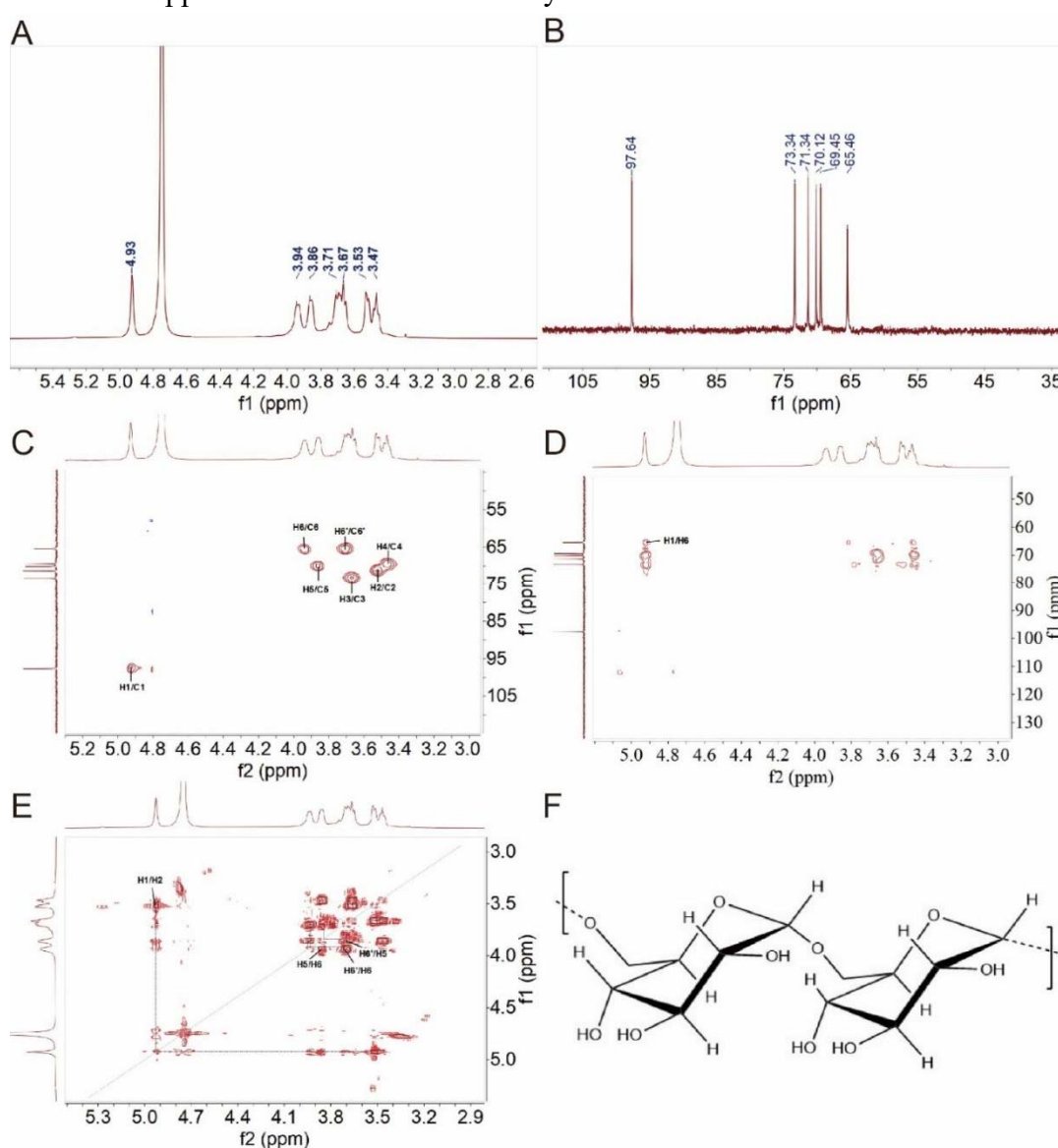


Fig. 3 NMR-based structural elucidation of EPS. (A) ^1H , (B) ^{13}C , (C) HSQC, (D) HMBC, (E) COSY spectra, and (F) proposed repeating unit.

3.4.5. Surface microstructure of EPS

SEM revealed a porous microstructure of EPS, highlighting its potential role in functional properties. The EPS microstructure (irregular, sheet-like porous) was shown in Fig. 4A, contrasting with the smooth, dense

surface observed at 1000 $\times$ in Fig. 4B. The EPS microstructure resembled that of EPS from *W. cibaria* YB-1^[43]. EPS from different sources exhibit distinct microstructures, which may be due to distinct monosaccharides and linkage patterns^[44, 45].

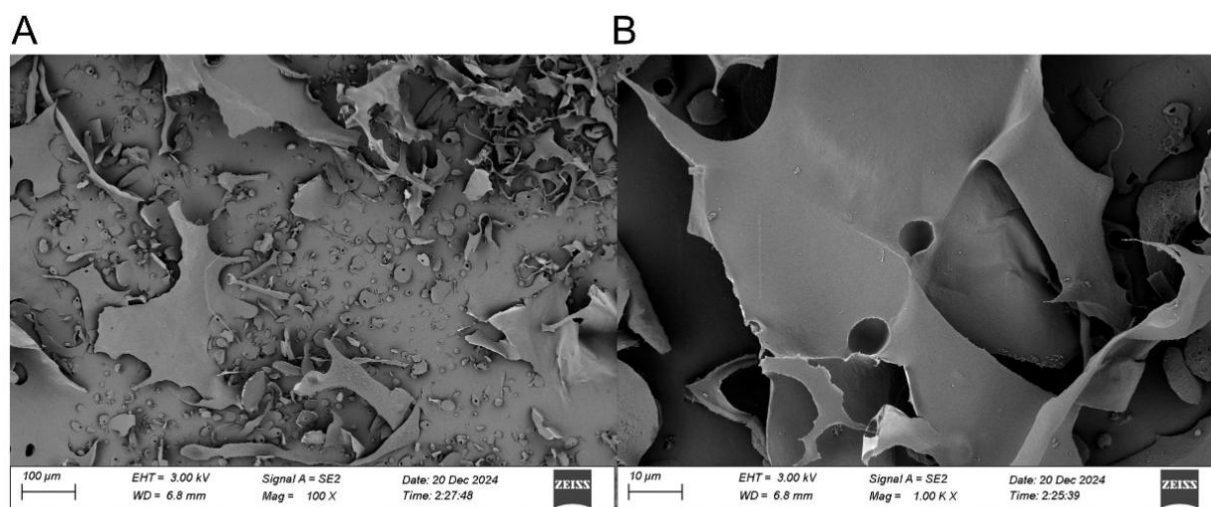


Fig. 4 Surface microstructure of EPS observed by SEM at 100 $\times$ (A) and 1000 $\times$ (B).

3.5. Physicochemical and antioxidant characteristics of EPS

3.5.1. Thermal behavior and stability

EPS thermal properties are critical for ensuring their functionality in food, pharmaceutical, and other industrial applications, especially under high-temperature processing conditions. The TGA curve reflects the mass change of EPS as a function of temperature, providing insight into the thermal stability and decomposition behavior. As shown in Fig. 5, the thermal degradation of EPS occurs in three distinct stages. During the first stage (17.34 $^{\circ}\text{C}$ to 125.55 $^{\circ}\text{C}$), a mass loss of 13.36% was observed, which is attributed to the cleavage of bonds between carboxyl groups and water molecules, leading to the release of bound water. The second stage (125.55 $^{\circ}\text{C}$ to 528.13 $^{\circ}\text{C}$) exhibited a mass loss of 67.25%, with a maximum weight loss rate of 11.82%/min, resulting from depolymerization of the EPS main chain. In the third stage, starting around 528.13 $^{\circ}\text{C}$, a further mass loss of 4.43% occurred, leaving 14.96% of residual material. This final stage is associated with the thermal degradation of the polymer backbone and subsequent reactions of the residual compounds.

The DTG curve exhibited a sharp peak at 308.56 $^{\circ}\text{C}$, corresponding to the thermal degradation temperature of the EPS, where the weight loss rate reaches its maximum. This value, corresponding to the decomposition temperature (T_d), was directly determined from the DTG peak. The DSC curve exhibited a broad endothermic peak at 81.36 $^{\circ}\text{C}$. Considering the amorphous structure of the EPS revealed by XRD, this peak is not a melting point, corresponds to the dehydration process (evaporation of bound water). The decomposition temperature of DRP105 EPS (308.56 $^{\circ}\text{C}$) is comparatively higher than that reported for *L. plantarum* KF5 EPS (279.59 $^{\circ}\text{C}$)^[46]. Such thermal stability is influenced by the EPS's complex molecular structure, monosaccharide composition and M_w ^[47]. Taken together, the absence of a distinct melting endotherm in the DSC thermogram and the broad amorphous halo in the XRD pattern indicate an amorphous

structure^[48]. Although the sample lacked a crystalline structure, it demonstrated notable thermal stability, which is likely attributed to the high Mw and dense hydrogen-bonding network^[49]. The good thermal stability of EPS laid the foundation for high-temperature-resistant materials^[41].

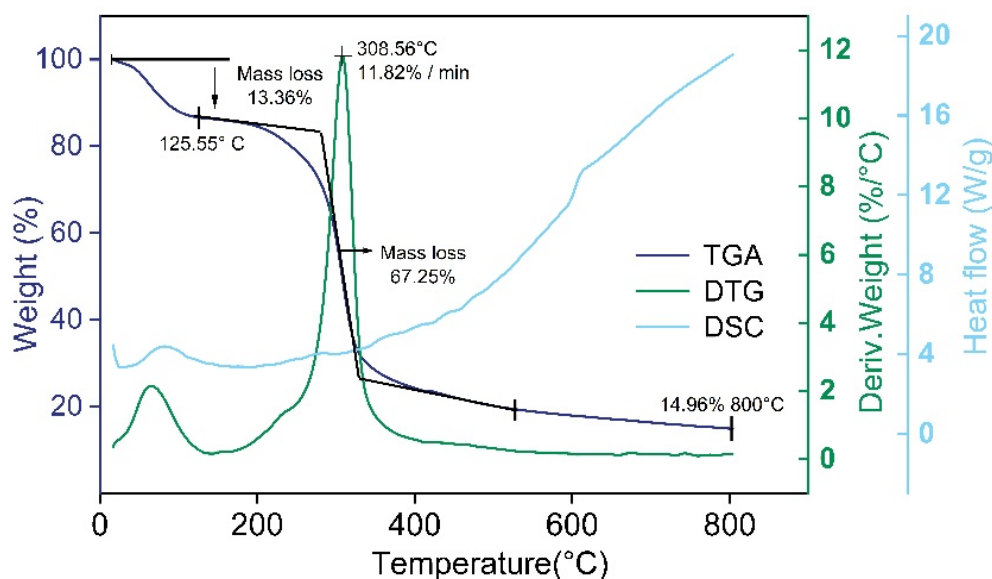


Fig. 5 Thermal properties of EPS characterized by TGA, DTG, and DSC analyses.

3.6. Dynamic changes in metabolomic profiles during fermentation

3.6.1 Multivariate statistical analysis

To elucidate flavor substance variations and bacterial metabolic pathway shifts during goldenberry fermentation, metabolite profiles were analyzed. A total of 2,541 metabolites were identified throughout the fermentation process. PCA, an unsupervised method, was used to visualize group clustering without prior sample designation. The PCA score plot (Fig. 6A) revealed clear separation between 0 h and 60 h along the first principal component (PC1), which accounted for 34.2% of the total variance. For enhanced discrimination, PLS-DA was conducted. The PLS-DA score plot demonstrated distinct separation between 0 h and 60 h, indicating significant temporal changes in goldenberry composition (Fig. 6B). Differential components were screened based on VIP score > 1. To visualize differentially abundant metabolites, a volcano plot (Fig. 6C) was generated using $\log_2\text{FC}$ and p-values, highlighting upregulated and downregulated metabolites. VIP scores derived from the PLS-DA model were used to identify screen significant variables, with $\text{VIP} > 1$ and $P < 0.05$ set as significance thresholds. After filtering, 39 differential metabolites were identified, comprising 31 downregulated and 8 upregulated compounds, reflecting dynamic changes in goldenberry juice during fermentation (Fig. 6D). These metabolites were classified into seven categories (Fig. 8E): organic acids (12), flavonoids and derivatives (9), amino acids and derivatives (6), phenolic compounds (2), nucleotides and nucleosides (5), purine derivatives (2), and others (3).

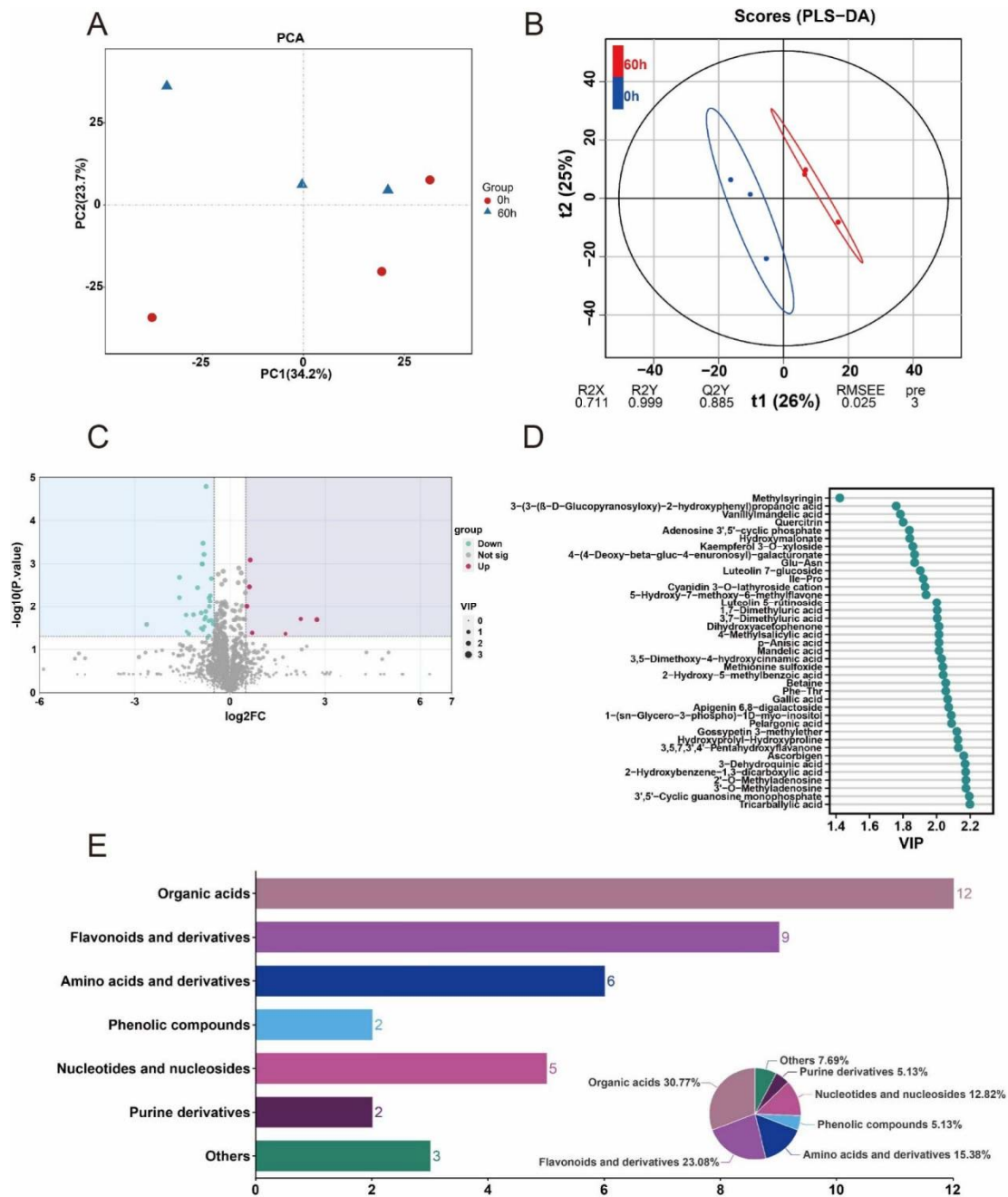


Fig. 6 Comprehensive metabolomic analysis of fermentation broth between 0 h and 60 h timepoints. (A) PCA, (B) PLS-DA, (C) Volcano plot of differential metabolites, (D) VIP score ranking, (E) Classification and distribution of differential metabolites.

3.6.2. Alterations in organic acids and derivatives

A more detailed analysis was conducted using $\log_2FC$ values to evaluate the variations. As shown in Fig. 7 (A, B), among organic acids, the upregulated substances included gallic acid, 3-dehydroquinic acid, vanillylmandelic acid, and tricarballic acid. Gallic acid is a type of polyphenol that exhibits anticancer, anti-inflammatory, antimicrobial, antipigmentogenic, antiviral, antiallergic, neuroprotective, and hepatoprotective activities^[50]. *L. mesenteroides* DRP 105 could metabolize phenolic acids present in goldenberries through decarboxylases and reductases, thereby producing gallic acid^[51]. The downregulated substance was 2-hydroxybenzene-1,3-dicarboxylic acid, which could be degraded to function as a supply of carbon and energy.

3.6.3. Dynamic changes of flavonoids and phenolic compounds

During the fermentation process, the vast majority of flavonoids and derivatives exhibited an overall decreasing trend (Fig. 7C). including compounds such as luteolin 5-rutinoside, luteolin 7-glucoside, kaempferol 3-O-xyloside, and quercitrin. LAB produced glycosidases such as β -glucosidase and α -rhamnosidase, which could hydrolyze the glycosidic moieties of flavonoid glycosides, thereby releasing free flavonoid aglycones. For example, quercitrin, the glycosylated form of quercetin, has diverse biological functions, including antioxidative stress, anti-inflammation, and antimicrobial activities^[52]. During fermentation, quercitrin was enzymatically hydrolyzed to quercetin, a bioactive aglycone known for its anti-inflammatory and immunomodulatory properties. Compared with glycosides, aglycones are more hydrophobic and can cross epithelial cells through passive diffusion^[53]. Luteolin and luteolin 7-glucoside exhibit antioxidant, anti-inflammatory, anticancer, anti-remodeling, and antihypertensive activities. Additionally, luteolin has shown potential in combating aging^[54]. Apigenin 6,8-digalactoside was downregulated to 0.33-fold, possibly due to enzymatic hydrolysis yielding the more bioactive aglycone apigenin.

Notably, 3,5,7,3',4'-pentahydroxyflavanone showed a particularly significant decline (0.16-fold). 3,5,7,3',4'-Pentahydroxyflavanone, commonly known as taxifolin, is a flavanone with multiple hydroxyl groups that contribute to its antioxidant activity. Taxifolin exhibits inhibitory effects on inflammation, cancer, microbial infections, oxidative stress, as well as cardiovascular and liver diseases^[55, 56]. The synergistic action of multiple microbial enzyme systems might have converted taxifolin in goldenberries into glycosylated derivatives or other flavonoids by activating specific metabolic pathways. Gossypetin 3-methylether was the only metabolite that increased significantly (1.40-fold). Gossypetin is a natural flavonoid with antioxidant, antibacterial, anticancer, and anti-inflammatory activities^[57, 58]. Structurally, the methylation of flavonoids reduces the number of free hydroxyl groups, thereby increasing their lipophilicity. This structural modification is often associated with enhanced membrane permeability and metabolic stability compared to their parent aglycones or glycosides, as supported by previous pharmacokinetic studies^[59]. Previous studies have shown that methylating flavonoids increases their metabolic stability and enhances membrane transport, leading to improved absorption and significantly higher oral bioavailability^[60]. The structural features of flavonoids are closely linked to their health-promoting effects, as they govern their capacity to chelate metal ions, scavenge reactive oxygen species (ROS), and interact with biological targets to elicit specific biological responses^[61, 62]. During this process, EPS may slow down its oxidative degradation by embedding or hydrogen bonding with flavonoids. Flavonoid aglycones and EPS jointly enhance the antioxidant and functional properties of fermentation products^[63]. In conclusion, flavonoids showed a general decreasing trend during the fermentation of goldenberries by *L. mesenteroides* DRP105, with taxifolin exhibiting a particularly notable decline, consistent with the findings of studies on lactic acid fermentation of fig (*Ficus carica*) fruit flavonoids^[64]. *L. mesenteroides* DRP105 biotransformed flavonoids in goldenberries into more stable, bioavailable derivatives with potential health benefits. Numerous studies have found that flavonoid glycosides

in fruits (such as rutin and hesperidin) are easily hydrolyzed by β -glucosidase of LAB and converted into aglycosides (such as quercetin and apigenin), thereby enhancing their bioavailability. Moreover, the strain modifies the flavonoid structure through enzymatic reactions (such as converting catechins into epicatechins), thereby altering its antioxidant activity. In a few cases, flavonoids may be further degraded into phenolic acids or polymerized into complex polyphenols.

Ascorbigen (ABG) increased significantly (1.54-fold) during the fermentation process. Goldenberries are rich in ascorbic acid, and the acidic environment generated during fermentation by *L. mesenteroides* DRP105 facilitated the conversion of glucosinolates or other indole-containing derivatives, along with ascorbic acid, into ascorbigen^[65, 66]. It has been reported that ABG possesses anticarcinogenic effects, possibly due to a reduction in the estrogen pool, which reduces the formation of 16 α -OHE1^[67], and it also exhibits immunomodulatory activity^[68]. Throughout the fermentation process, amino acids and their derivatives functioned as key nitrogen sources for microbial proliferation, while phenolic compounds might have undergone metabolic conversion into simpler phenolic acids or been reduced to aromatic alcohols.

Preliminary analysis indicated that *L. mesenteroides* DRP105 metabolized goldenberry sugars to produce organic acids. Flavonoids, abundant in goldenberry, were biotransformed into more bioavailable derivatives by the bacterium. Phenolic compounds, amino acids, and their derivatives likely supported bacterial growth and metabolic activity. The other category included ascorbigen, 3-(3-(β -D-glucopyranosyloxy)-2-hydroxyphenyl) propanoic acid, and methylsyringin. Notably, amino acid derivatives, purines, and nucleosides appeared to regulate fermentation, whereas organic acids and flavonoid derivatives contributed to the enhanced bioactivity (antioxidant and anti-inflammatory effects) of fermented juice^[69].

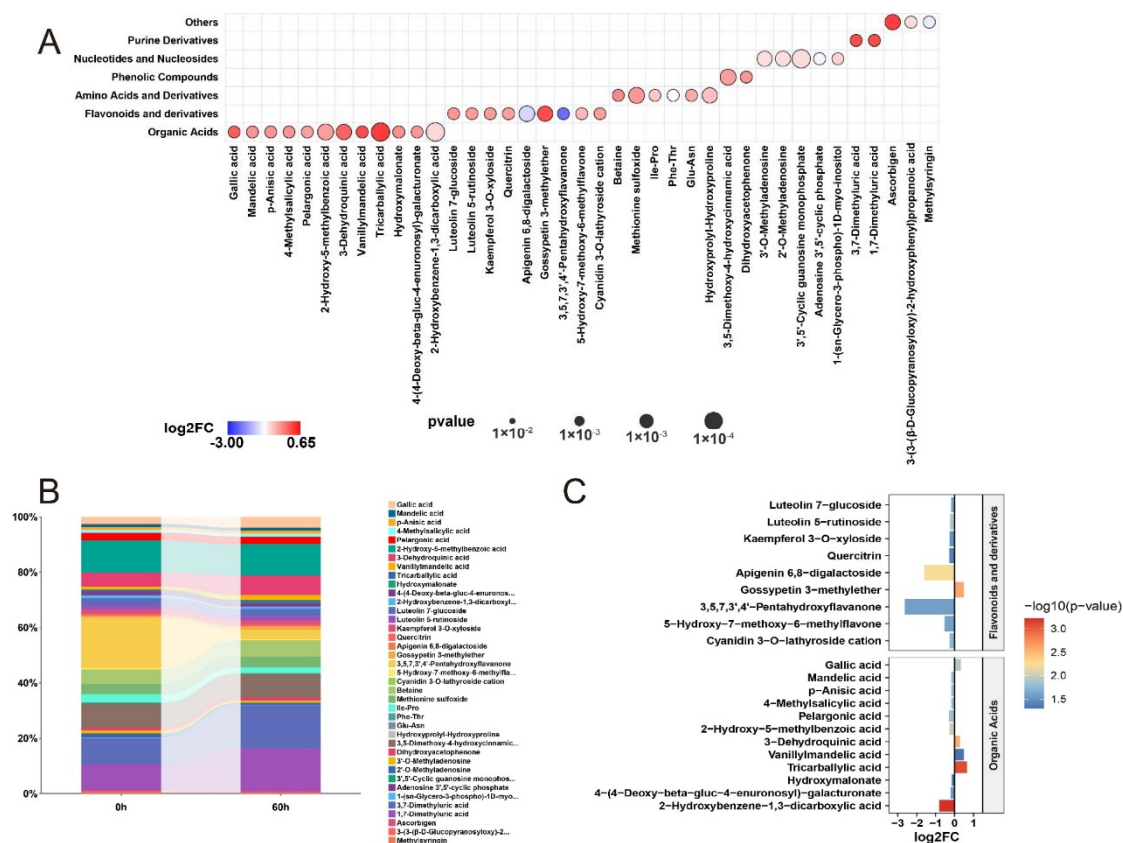


Fig. 7 Variation of significant metabolites between 0 h and 60 h of fermentation. (A) Bubble heatmap displaying log₂FC and p-values of significantly altered metabolites, (B) Percentage-stacked bar chart illustrating the relative abundance of key metabolites between 0 h and 60 h, (C) Bar plots showing the log₂FC of two types.

3.7. Spectroscopic and structural evidence of the interaction

3.7.1. FT-IR spectral changes

FT-IR spectral analysis of the EPS (Fig. 8A) showed characteristic absorption bands associated with its functional groups. A broad band at 3435.93 cm⁻¹ was attributed to O-H stretching vibrations^[41]. The peak observed at 2929.78 cm⁻¹ corresponded to C-H stretching vibrations of the sugar ring. A signal at 1639.12 cm⁻¹ indicated the presence of bound water^[37]. Peaks in the region of 1000–1200 cm⁻¹ are consistent with carbohydrate ring vibrations. Free taxifolin showed an O–H band at 3429.94 cm⁻¹ and a characteristic band at 1631.67 cm⁻¹, together with multiple fingerprint peaks in the 1600–1000 cm⁻¹ region^[70]. Notably, after complexation, the O–H stretching band of the EPS–taxifolin complex shifted to a lower wavenumber (3423.68 cm⁻¹) and became broader relative to those of free EPS and taxifolin, suggesting strengthened hydrogen bonding between hydroxyl groups of EPS and phenolic hydroxyl (and carbonyl-associated) groups of taxifolin.^[71, 72] In addition, noticeable changes in the 1630–1640 cm⁻¹ region suggesting an altered microenvironment^[72, 73]. Meanwhile, several characteristic bands of taxifolin in the 1600–1000 cm⁻¹ region became less resolved and weakened in the complex, which can be attributed to its relatively low proportion and overlap with the strong polysaccharide fingerprint bands of EPS, suggesting restricted molecular vibrations due to embedding within the EPS matrix^[74]. Overall, the spectral changes support the formation of an EPS–taxifolin complex mainly driven by non-covalent interactions, particularly hydrogen bonding.

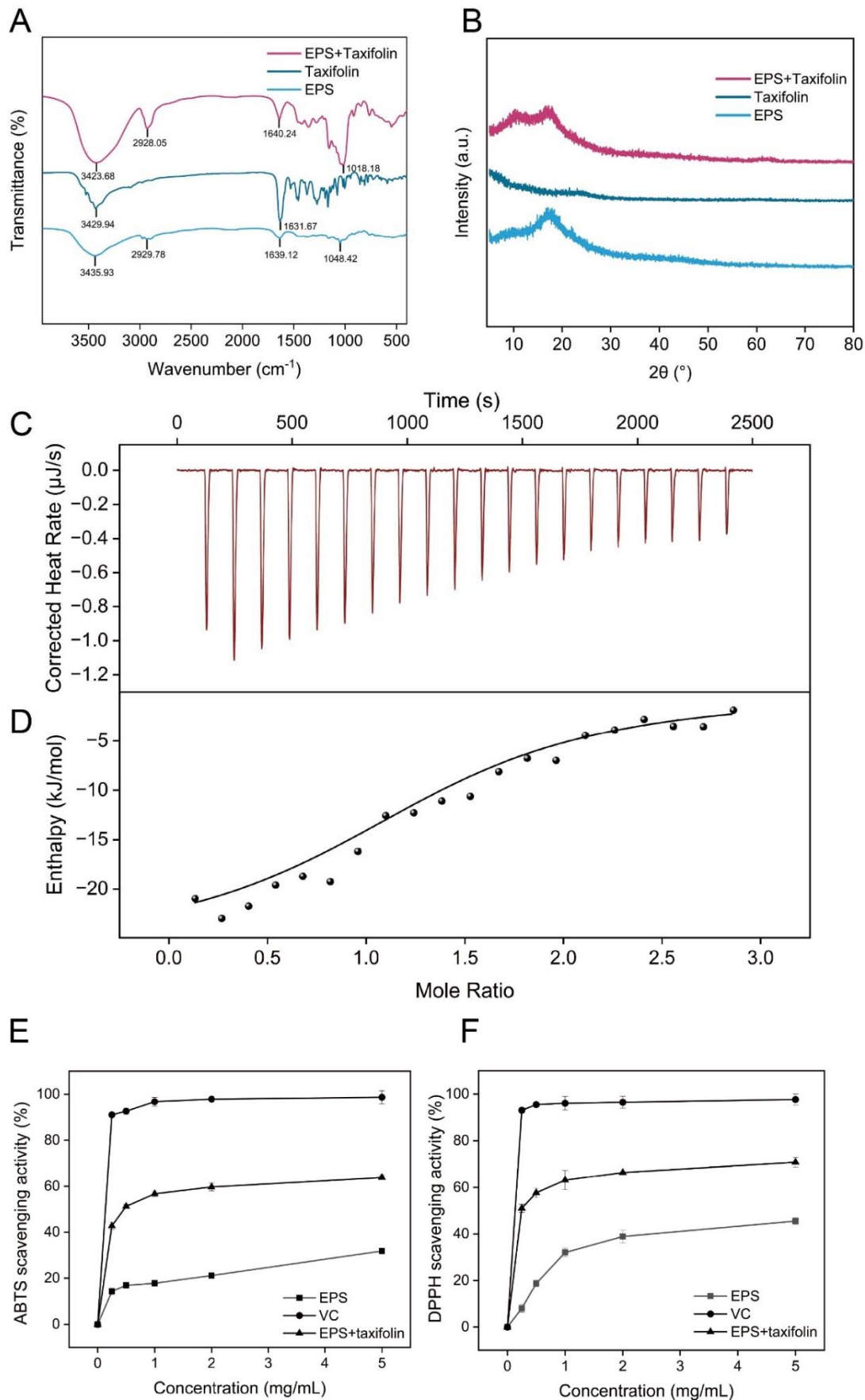


Fig. 8 EPS–taxifolin interaction and antioxidant performance. (A) FT-IR spectra of EPS, taxifolin, and the EPS–taxifolin complex, (B) XRD patterns of EPS, taxifolin, and the EPS–taxifolin complex, (C) Raw heat flow thermograms for titrating EPS into taxifolin, with dilution heat corrected using the solvent control, (D) Dilution-corrected integrated heats plotted versus molar ratio and fitted with an independent binding model, (E) ABTS radical scavenging activity of EPS, taxifolin, and the EPS–taxifolin complex, (F) DPPH radical scavenging activity of EPS, taxifolin, and the EPS–taxifolin complex.

3.7.2. Crystalline structure

The physical states of EPS, taxifolin, and the EPS-taxifolin complex were characterized by X-ray diffraction, as shown in Fig. 8B. The diffractogram of pure EPS (Black line) exhibited a broad in the 2θ range of $15\text{--}20^\circ$, with no sharp diffraction peaks observed. This suggests that the EPS predominantly exhibits an amorphous and non-crystalline structure. The broad, diffuse peak is characteristic of amorphous materials, reflecting a lack of long-range periodicity in the molecular packing of the EPS^[75]. Consistent with previous studies, XRD profiles of LAB EPS typically show a “hump-like” pattern, further supporting the predominance of an amorphous phase^[45, 76]. A broad peak at 19° (2θ) was observed in the EPS of *W. cibaria* HDL-4, which was attributed to disordered aggregation of polysaccharide chains^[77]. The XRD pattern of the EPS–taxifolin complex (blue line) preserved the broad amorphous halo characteristic of the pure EPS matrix. Notably, the characteristic sharp crystalline reflections of pure taxifolin completely disappeared in the complex. These features indicate that the complexation did not generate a new crystalline phase, suggesting that taxifolin was dispersed in a non-crystalline (amorphous) state within the EPS carrier^[78]. Although a weak reflection near $2\theta \approx 10^\circ$ was observed for the complex, the absence of multiple sharp diffraction peaks implies that this feature is likely associated with short-range ordering induced by intermolecular interactions rather than the formation of a crystalline phase^[79]. Maintaining this predominantly amorphous state is highly beneficial for poorly water-soluble flavonoids, as it is often correlated with improved apparent solubility and faster dissolution rates compared to their crystalline counterparts^[80].

3.7.3. Thermodynamic parameters

ITC is a powerful and widely used technique for the direct characterization of interactions between polyphenols and macromolecules, which was employed to study the interaction between EPS and taxifolin. ITC revealed a clear exothermic interaction between EPS and taxifolin. The heat released per injection decreased progressively and approached saturation with increasing molar ratio after subtraction of dilution heats from the solvent control, consistent with a saturable binding process. As shown in Fig. 8C and 8D, fitting the integrated heats to an independent binding model yielded $K_d = 2.66 \mu\text{M}$, $\Delta H = -28.72 \text{ kJ/mol}$, $\Delta S = 10.41 \text{ J/mol}\cdot\text{K}$, and an apparent stoichiometry of $n = 1.319$. Accordingly, the binding free energy at 298 K was $\Delta G \approx -31.8 \text{ kJ/mol}$, indicating a spontaneous association^[81]. Comparison of thermodynamic contributions showed that binding was predominantly enthalpy-driven, suggesting that hydrogen bonding and specific intermolecular contacts play a major role^[82], while the modest favorable entropy likely reflects partial desolvation and release of ordered water molecules upon complex formation. Above all, the ITC and FT-IR results confirm a direct interaction between taxifolin and EPS, which is predominantly mediated by hydrogen bonding involving hydroxyl groups on both molecules and is consistent with the exothermic, enthalpy-driven binding profile observed by ITC. Consistent with previous studies, such polysaccharide–flavonoid associations are frequently attributed to hydrogen bonding between hydroxyl-rich functional groups, further impacting their potential health effects^[83].

3.7.4. Synergistic antioxidant effect between EPS and taxifolin

(1) ATBS scavenging activity

The ATBS scavenging activity of both EPS and VC exhibited a concentration-dependent increase (Fig. 8E). EPS exhibited a rapid increase in ABTS scavenging activity, reaching 14.33% at 0.5 mg/mL, followed by a slower increase, reaching 31.79% at 5 mg/mL. In contrast, VC exhibited a much higher activity, achieving 91.07% scavenging at 0.25 mg/mL and reaching 98.67% at 5 mg/mL. EPS has moderate antioxidant activity. EPS from different LAB vary in monosaccharide composition leading to differences in ABTS radical-scavenging capacity. EPS from *L. fermentum* often exhibit stronger scavenging activity^[84]. Low Mw EPS more readily interact with ABTS radicals, showing higher scavenging efficiency, whereas high Mw EPS may be sterically hindered, unless enriched in reactive groups (e.g., hydroxyl, carboxyl), which can still confer high activity^[85]. The EPS–taxifolin interaction systems showed substantially higher ABTS scavenging at all EPS concentrations, rising from $42.73 \pm 1.51\%$ (0.25 mg/mL) to $51.23 \pm 0.80\%$ (0.5 mg/mL), $56.67 \pm 1.12\%$ (1.0 mg/mL), $59.67 \pm 1.69\%$ (2.0 mg/mL), and reaching $63.78 \pm 0.87\%$ (5.0 mg/mL). Therefore, at 5.0 mg/mL EPS, the presence of taxifolin increased ABTS scavenging by approximately 100.63% relative to EPS alone $31.79 \pm 1.01\%$.

(2) DPPH scavenging activity

The DPPH radical scavenging activity of EPS exhibited a dose-dependent increase (Fig. 8F). The scavenging rate reached 32.02% at 1.0 mg/mL and gradually increased to 45.51% at 5.0 mg/mL. In contrast, the positive control (VC) exhibited significantly higher activity, reaching 97.69% at 5 mg/mL. Overall, the isolated EPS demonstrated moderate antioxidant activity. The DPPH radical scavenging activity of EPS was higher than that of *L. plantarum* KX041 EPS-2-1 at a concentration of 6 mg/mL^[86]. A previous study reported that ST-EPS1 from *Streptococcus thermophilus* CGMCC 7.179 achieved 47.11% scavenging at 10.0 mg/mL^[87]. Such differences may be attributable to variations in monosaccharide composition or Mw, as EPS enriched in reducing sugars (e.g., glucose and galactose) or acidic sugars, and those containing hydroxyl, carboxyl, or sulfate groups, can effectively donate hydrogen atoms even at low concentrations^[85, 88]. In contrast, the EPS–taxifolin systems consistently exhibited higher scavenging capacities at the same EPS concentrations, increasing from $51.03 \pm 1.71\%$ (0.25 mg/mL) to $57.71 \pm 2.08\%$ (0.5 mg/mL), $63.16 \pm 4.20\%$ (1.0 mg/mL), $66.44 \pm 0.34\%$ (2.0 mg/mL), and $70.80 \pm 2.03\%$ (5.0 mg/mL). Thus, at 5.0 mg/mL EPS, incorporating a constant amount of taxifolin enhanced the DPPH scavenging by approximately 25% compared with EPS alone 55.57%.

Enhanced antioxidant activity upon polysaccharide–phenolic association/complexation has been widely reported in food systems, mainly driven by non-covalent interactions such as hydrogen bonding^[89]. The enhanced antioxidant activity is more likely due to EPS–taxifolin interactions, mainly hydrogen bonding, as supported by the O–H band red-shift/broadening in FT-IR (to 3423.68 cm^{-1})^[12]. Therefore, EPS acts not only as an antioxidant itself but also as a functional matrix that enhances the apparent radical-scavenging performance of taxifolin.

4. Conclusion

This research delineated the structure and characteristics of an EPS synthesized by *L. mesenteroides* DRP105 cultivated on a goldenberry substrate, and profiled the metabolic shifts during its biosynthesis. The EPS was characterized as a dextran with α -(1→6)-linked glucose units, and exhibited an irregular, sheet-like, and porous morphology. It also demonstrated favorable thermal stability and antioxidant activity. Notably, metabolite profiling revealed that *L. mesenteroides* DRP105 biotransforms goldenberry-derived compounds, particularly flavonoids and their derivatives, into more stable and bioavailable forms with potential health benefits, and EPS interacts with taxifolin to form a noncovalent complex, which enhances the antioxidant activity of EPS. In conclusion, this study provides the basis for the application of EPS and offers valuable insights into developing functional foods enriched with bioactive compounds. Future studies should focus on optimizing fermentation conditions (such as temperature, pH, and inoculum size) to maximize the yield of both EPS and bioactive aglycones. Furthermore, selecting more logical and frequent time points for metabolomic analysis will be crucial to map the precise kinetic trajectory of flavonoid biotransformation and to elucidate the dynamic metabolic mechanisms of *L. mesenteroides* DRP105.

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors have declared no conflicts of interest.

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