International Journal of Biological Macromolecules 146 (2020) 508–517

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Polysaccharides from loquat (*Eriobotrya japonica*) leaves: Impacts of extraction methods on their physicochemical characteristics and biological activities

Yuan Fu [a,1], Fen Li [a,1], Ye Ding [a], Hua-Yu Li [a], Xian-Rong Xiang [a], Qiong Ye [a], Jian Zhang [a], Li Zhao [a], Wen Qin [a], Ren-You Gan [b,*], Ding-Tao Wu [a,*]

[a] Institute of Food Processing and Safety, College of Food Science, Sichuan Agricultural University, Ya'an 625014, Sichuan, China
[b] Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu 610213, China

ARTICLE INFO

Article history:
Received 20 December 2019
Received in revised form 27 December 2019
Accepted 31 December 2019
Available online 7 January 2020

Keywords:
Loquat
Extraction method
Polysaccharide
Chemical structure
Bioactivity

ABSTRACT

Impacts of hot water extraction (HWE), pressurized water extraction (PWE), high-speed shearing homogenization extraction, microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), ultrasound assisted enzymatic extraction, and ultrasound-microwave assisted extraction (UMAE) on physicochemical characteristics and bioactivities of polysaccharides from loquat (*Eriobotrya japonica*) leaves (LLPs) were investigated. Results showed that the degrees of esterification, contents of phenolics and uronic acids, constituent monosaccharides, apparent viscosities, and molecular weights of LLPs varied by different extraction methods. Bioactivities of LLPs were also significantly affected by different extraction methods. The high molecular weight and high degree of esterification of LLP-W and LLP-P extracted by HWE and PWE, respectively, might contribute to their strong binding capacities. The strong antioxidant activities and inhibitory effects on α-amylase and α-glucosidase were found in LLP-M and LLP-U extracted by MAE and UAE, respectively, which might be attributed to their contents of uronic acids, contents of total phenolics, and molecular weights. The low molecular weights and viscosities of LLP-U and LLP-UM extracted by UMAE might contribute to their strong prebiotic effects. These findings could provide scientific foundations for selecting appropriate extraction methods to obtain LLPs with desired bioactivities for applications in the pharmaceutical and functional food industries.

1. Introduction

Loquat (*Eriobotrya japonica* L.) is a semitropical fruit tree widely distributed in Southeastern China, which is classified as the Rosaceae family [1]. In China, loquat leaves are highly requested as a prevalent raw material of tea and an important traditional Chinese medicine [2–4]. Extracts of loquat leaves have been proved to possess various biological activities [5,6], such as anti-inflammatory [7], hepatoprotective [8,9], antidiabetic, anti-obesity [10], and antioxidant effects [11]. Moreover, previous studies have demonstrated that polysaccharides which richly distribute in loquat leaf have superlative *in vitro* anti-obesity and antidiabetic effects [12]. Hence, polysaccharides from loquat leaves (LLPs) have potential applications in the pharmaceutical and functional food industries.

Extraction processes are essential on the application of natural polysaccharides in the pharmaceutical and functional food industries, which have great effects on the extraction yields, physicochemical properties, and biological activities of natural polysaccharides [13,14]. Hot water extraction (HWE) is the simple and convenient method for the extraction of natural polysaccharides [14,15]. However, the drawbacks of HWE contain high extraction temperature, long extraction time, and low extraction efficiency [16,17]. Currently, the extraction techniques, including microwave assisted extraction (MAE) [18,19], pressurized water extraction (PWE) [20,21], high-speed shearing homogenization extraction (HSHE) [22–24], ultrasonic assisted extraction (UAE) [25,26], ultrasonic assisted enzymatic extraction (UAEE) [27–29], and ultrasound-microwave assisted extraction (UMAE) [30,31], are carried out to extract polysaccharides from natural resources. Compared with the HWE, the extraction efficiency of MAE, PWE, HSHE, UAE, UAEE, and UMAE is much higher [32]. MAE is environmental friendly in terms of its reduced use of energy and solvents, which accelerate the mass transfer of target compounds by using its microwave energy [18]. The high pressure and temperature of PWE improve the solubility of object compounds, allowing them to penetrate better into the sample matrix [20,21,33]. HSHE is an extraction technique with high efficiency, low extraction temperature, less energy consumption, and short

* Corresponding authors.
E-mail addresses: ganrenyou@caas.cn (R.-Y. Gan), DT_Wu@sicau.edu.cn (D.-T. Wu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijbiomac.2019.12.273
0141-8130/

extraction time [23]. UAE as an energy efficient method gives high extraction yield, which is fast and requires less solvent [29]. At present, the combination of ultrasound with microwave (UMAE) or enzyme (UAEE) has also been carried out to improve the extraction efficiency of polysaccharides from natural resources [23,27,28,31]. However, the impacts of different extraction methods on the physicochemical characteristics and bioactivities of LLPs have never been investigated. It is unknown that whether the physicochemical characteristics and bioactivities of LLPs are affected by different extraction methods.

Hence, for the purpose of evaluating the impacts of different extraction methods on physicochemical characteristics and bioactivities of LLPs, seven extraction methods, including HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, were applied to extract polysaccharides from loquat leaves. Then chemical characteristics, rheological properties, *in vitro* antioxidant activities, inhibitory effects on α-amylase and α-glucosidase, binding capacities, and prebiotic activities of LLPs obtained by different extraction methods were compared. Results from this study will give scientific foundations for selecting appropriate extraction methods for obtaining LLPs with the relatively high bioactivities, which will expand their potential applications in the pharmaceutical and functional food industries.

2. Material and methods

2.1. Material and reagents

2.1.1. Chemicals and reagents

Loquat leaves (*Eriobotrya japonica* cv. *Chuannong*8) were purchased from Chengdu, Sichuan Province, China. Loquat leaves were cut into small pieces, and then dried by microwave at 400 W, and pulverized. The 60 mesh sieve was used to screen the powder, and then the powder was stored at −20 °C.

2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), α-glucosidase (10 U/mg), soluble starch, α-amylase (1000 U/mg), acarbose, griess reagent, 1-phenyl-3-methyl-5-pyrazolone (PMP), vitamin C, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and 4-nitrophenyl β-D-glucopyranoside (pNPG) were bought from Sigma-Aldrich (St. Louis, MO, USA). The free cholesterol assay kit was bought from Solarbio (Beijing, China). Other chemicals utilized in this work were of analytical grade.

2.1.2. Bacterial strains

Three *Lactobacillus* strains, including *L. rhamnosus* CICC 6133, *L. rhamnosus* CICC 6151, and *L. acidophilus* CICC 6089, were bought from China Center of Industrial Culture Collection, and cultivated according to a method reported formerly [34].

2.2. Preparation of LLPs by different extraction methods

2.2.1. Hot water extraction

HWE was performed based on our experiment reported formerly with slight modifications [12]. Briefly, 1.0 g of sample was firstly refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, 30.0 mL of deionized water was added into the extraction residue for the further extraction of polysaccharides at 95 °C for 2 h. Then the protein in the crude extracts was removed by pancreatin (1 U/mL). Three volumes of 95% (v/v) ethanol were added to precipitate crude polysaccharides. After centrifugation, the crude polysaccharides were washed with 70% (v/v) ethanol. Finally, LLPs obtained by HWE were freeze dried and coded as LLP-W.

2.2.2. Microwave assisted extraction

MAE was also carried out by the formerly optimized method with slight revisions [26]. In brief, 1.0 g of sample was firstly refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, 40.0 mL of deionized water was added into the extraction residue for the further extraction of polysaccharides by utilizing a microwave oven (MKJ-J1-3, Qingdao Makewave Microwave Applied Technology Co., Ltd., Shandong, China) at 500 W for 6.5 min. Finally, after the treatment procedures as described in Section 2.2.1, LLPs extracted by MAE were freeze dried and coded as LLP-M.

2.2.3. Pressurized water extraction

PWE was carried out by the previously reported method with slight revisions [20]. In brief, 1.0 g of sample was firstly refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, 30.0 mL of deionized water was added into the extraction residue for the further extraction of polysaccharides by utilizing a laboratory-scale high pressure reactor (LEC-300, Shanghai Laibei Scientific Instruments Co., Ltd., Shanghai, China) at 55 °C and 1.8 MPa for 40 min. Finally, after the treatment procedures as described in Section 2.2.1, LLPs extracted by PWE were freeze dried and coded as LLP-P.

2.2.4. High-speed shearing homogenization extraction

HSHE was carried out based on the optimized experiment with slight modifications [23]. In brief, 1.0 g of sample was firstly refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, the extraction residue was further mixed with 50.0 mL of deionized water, and extracted by using a high-speed shearing homogenization (AD500S-H, ANGNI Instruments Co., Ltd., Shanghai, China) at 7500 rpm for 10 min at room temperature. After the treatment procedures as described in Section 2.2.1, LLPs extracted by HSHE were obtained and coded as LLP-HSH.

2.2.5. Ultrasonic assisted extraction

UAE was performed by the optimized method with slight revisions [26]. In brief, 1.0 g of sample was firstly refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, the extraction residue was further extracted with 40.0 mL of deionized water by utilizing an ultrasonic homogenizer (JY92-IIN, Ningbo Scientz Biotechnology Co., Ltd., China) at 450 W for 20 min. Finally, after the treatment procedures as described in Section 2.2.1, LLPs extracted by UAE were obtained and coded as LLP-U.

2.2.6. Ultrasonic assisted enzymatic extraction

UAEE was performed by an optimized experiment with some modifications [35]. Firstly, 1.0 g of sample was refluxed with 10.0 mL of 80% (v/v) ethanol at 80 °C for 1 h. Then, the extraction residue was mixed with 40.0 mL of deionized water and 50.0 mg of cellulase, and extracted by using an ultrasonic homogenizer (JY92-IIN, Ningbo Scientz Biotechnology Co., Ltd., China) at 450 W for 20 min. Finally, after the treatment procedures as described in Section 2.2.1, LLPs extracted by UAEE were obtained and coded as LLP-UE.

2.2.7. Ultrasound-microwave assisted extraction

UMAE was carried out by the combination of ultrasound assisted extraction and microwave assisted extraction. The ultrasound assisted extraction was performed as described in Section 2.2.5. Then, the extraction mixture was further extracted by MAE as described in Section 2.2.2. Finally, after the treatment procedures as described in Section 2.2.1, LLPs extracted by UMAE were obtained and coded as LLP-UM.

2.3. Characterization of LLPs obtained by different extraction methods

2.3.1. Chemical components analysis

The proteins, uronic acids, total polysaccharides, and total phenolics in LLPs were measured by the Bradford's method, the *m*-hydroxydiphenyl method, the phenol-sulfuric acid method, and the Folin-Ciocalteu assay according to our previous studies [12,36], respectively.

2.3.2. Analysis of molecular weights and constituent monosaccharides

The absolute weight-average molecular weights (M_w) and polydispersities (M_w/M_n) of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were detected by high performance size exclusion chromatography equipped with the multi angle laser light scattering and refractive index detector (HPSEC-MALLS-RID, Wyatt Technology Co., Santa Barbara, CA, USA) [12]. Shodex OHpak SB-806 M HQ (300 mm × 8.0 mm, i.d.) column was used for the isolation of LLPs at 35 °C. In addition, constituent monosaccharides of LLPs were also analyzed by the high performance liquid chromatography (ThermoFisher, Waltham, MA, USA) equipped with a phenomenex gemini 5u C18 110A (150 mm × 4.6 mm, 5 μm) column based on the method reported formerly [12].

2.3.3. Fourier transform infrared spectroscopy analysis

The LLPs were grounded with KBr powder, depressed into a 1-mm pellet, and detected by a Nicolet iS 10 FT-IR (ThermoFisher scientific, Waltham, MA, USA) according to the method reported formerly [12].

2.4. Rheological measurements

Rheological properties of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were detected by the formerly reported method [37]. The rheological properties of LLPs were determined at the concentration of 0.4% (w/v) by using a Discovery Hybrid Rheometer-1 (DHR-1, TA instruments, New Castle DE, USA) with a parallel steel plate (40 mm diameter, 1.0 mm gap).

2.5. Determination of in vitro binding properties

The assay of *in vitro* binding capacities of LLPs was carried out by the formerly reported methods [12]. The fat binding capacities, cholesterol binding capacities, and bile acid binding capacities of LLPs were exhibited as gram of binding fat per gram of LLPs (g/g), milligram of binding cholesterol per gram of LLPs (mg/g), and a percent of blank control (%), respectively.

2.6. Determination of in vitro antioxidant activities

The ABTS, DPPH, nitric oxide (NO) scavenging activities, and reducing powers of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were detected by the formerly reported methods [20,38]. The LLPs were detected at five different concentrations against distilled water as blank control, and the BHT or vitamin C was utilized as the positive control. Afterwards, a log-regression curve was set up for calculating IC_{50} values (mg/mL).

2.7. Determination of in vitro α-amylase and α-glucosidase inhibitory effects

α-Amylase and α-glucosidase inhibitory effects of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were detected by the formerly reported methods [12]. The positive control was acarbose. The LLPs were detected at five different concentrations, and the calculation of IC_{50} values (μg/mL) was carried out by setting up a log-regression curve.

2.8. Determination of in vitro prebiotic activities

The *in vitro* prebiotic activities of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were detected by a modified method reported formerly [39]. In brief, the MRS broth containing 0.05% (w/v) L-cysteine without carbohydrates was utilized as the basal medium. Effects of LLPs on the growth of three *Lactobacilli* strains, including *L. rhamnosus* CICC 6133, and *L. rhamnosus* CICC 6151, *L. acidophilus* CICC 6089, were investigated. Inulin and the basal MRS were taken as the positive control and blank control, respectively. Each sample was dissolved in the MRS broth at the concentration of 0.5% (w/v). The concentration of each *Lactobacilli* strain was set as 1×10^7 CFU/mL. Both *L. rhamnosus* CICC 6151 and *L. acidophilus* CICC 6089 were incubated at 37 °C, and *L. rhamnosus* CICC 6133 was incubated at 30 °C for 24 h, respectively. Finally, the optical density values at 600 nm were determined.

2.9. Analysis of total short chain fatty acids (SCFAs)

The total SCFAs of the fermented broths were investigated by a method reported formerly [40]. Firstly, the fermented broths were centrifuged at 8000 ×*g* for 10 min, and the 400 μL of supernatants were mixed with 400 μL of internal standard (0.05 M of 2-ethylbutyric acid). Then each sample was analyzed by the Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) with a HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detection. The GC operation conditions were set as follows: the flow rate of nitrogen, 30 mL/min; the flow rate of air, 260 mL/min; the follow rate of hydrogen, 30 mL/min; the injection volume, 1 μL; the injection temperature, 250 °C; the detector temperature, 250 °C; the initial oven temperature, 100 °C for 1 min, next raised to 180 °C by 5 °C/min, then kept at 180 °C for 2 min.

2.10. Statistical analysis

All the data were displayed as the means ± standard deviations, and the experiments were performed in triplicate. The statistical significances were conducted by the analysis of variance (ANOVA) (SPSS software, version 24.0), followed by Duncan's test.

3. Results and discussions

3.1. Physicochemical characteristics of LLPs

3.1.1. Chemical compositions

The extraction yields and chemical compositions of polysaccharides extracted from loquat leaves by different extraction methods were expressed in Table 1. The extraction yields of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM ranged from 2.95% to 5.05%, which were close to the previous study (ranged from 3.62% to 5.29%) [12]. Results showed that the extraction yields of LLP-P, LLP-U, LLP-UE, and LLP-UM were significantly ($p < 0.05$) higher than that of LLP-W (Table 1), which suggested that the PWE, UAE, UAEE, and UMAE methods possessed higher extraction efficiency than that of HWE. The high pressure of PWE and the behavior of cavitation bubbles upon mass transfer from UAE, UAEE, and UMAE might increase the solvent diffusion rate and the solubility of target components, allowing them to penetrate better into the sample matrix to increase the extraction yields [13,14,34,40,41]. Moreover, the contents of total polysaccharides in LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM ranged from 81.67% to 89.42%, which were quiet close to the previous study (ranged from 81.80% to 83.07%) [12]. A few proteins were detected in LLPs, which ranged from 5.64% to 6.96%. Results showed that polysaccharides were the major bioactive compounds in LLPs. The contents of uronic acids in LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE and LLP-UM were determined to be 34.11%, 33.45%, 33.54%, 33.88%, 35.47%, 33.73%, and 34.37%, respectively, which were closed to the former study (ranged from 27.04% to 41.46%) [12]. It has been confirmed that the relatively high content of uronic acids in LLPs indicated the presence of pectin-like acidic polysaccharides in the loquat leaves [20,26,38]. The highest content of uronic acids was detected in LLP-U obtained by UAE among all tested samples. This phenomenon was also observed in some previous studies [13,19]. Furthermore, although most of phenolic compounds were taken away by ethanol extraction and ethanol precipitation, a few phenolic compounds were also detected in LLPs. The contents of total phenolic compounds in

Table 1
Chemical compositions of LLPs.

	LLP-W	LLP-M	LLP-P	LLP-HSH	LLP-U	LLP-UE	LLP-UM
Extraction yield (%)	2.95 ± 0.04^{c}	3.11 ± 0.05^{bc}	5.05 ± 0.49^{a}	3.16 ± 0.04^{bc}	4.53 ± 0.04^{abc}	4.73 ± 2.17^{ab}	4.93 ± 0.67^{a}
Total polysaccharides (%)	85.86 ± 0.35^{b}	85.82 ± 0.27^{b}	81.67 ± 0.38^{e}	82.52 ± 0.40^{d}	84.46 ± 0.50^{c}	89.42 ± 0.24^{a}	86.18 ± 0.26^{b}
Total uronic acids (%)	34.11 ± 0.81^{b}	33.45 ± 0.33^{b}	33.54 ± 0.26^{b}	33.88 ± 0.46^{b}	35.47 ± 0.21^{a}	33.73 ± 0.59^{b}	34.37 ± 0.85^{b}
Total phenolics (mg GAE/g)	15.43 ± 0.04^{f}	34.36 ± 0.01^{a}	25.53 ± 0.44^{d}	28.60 ± 0.06^{c}	29.61 ± 0.68^{b}	23.78 ± 0.05^{e}	28.82 ± 0.22^{c}
Total proteins (%)	6.56 ± 0.35^{a}	6.95 ± 0.13^{a}	5.64 ± 0.92^{a}	6.73 ± 0.17^{a}	6.96 ± 1.01^{a}	6.77 ± 0.32^{a}	6.91 ± 1.60^{a}
Degree of esterification (%)	17.78 ± 0.43^{bc}	15.80 ± 0.14^{c}	21.88 ± 0.92^{a}	18.15 ± 0.17^{b}	18.59 ± 1.01^{b}	18.37 ± 0.32^{b}	12.80 ± 0.17^{d}

LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively; Values represent mean ± standard deviation, and superscripts a–f differ significantly ($p < 0.05$) among LLPs.

LLPs ranged from 15.43 ± 0.04 to 34.36 ± 0.01 mg GAE/g, which indicated that LLPs might contain natural phenolic-polysaccharide conjugates [37]. Contents of total phenolic compounds in LLPs were also affected by different extraction methods. The lowest content of phenolic compounds was detected in LLP-W among all tested samples, which might be attributed to the degradation under the high temperature and long extraction time of HWE [42].

3.1.2. Molecular weights and constituent monosaccharides

Biological activities of polysaccharides are generally connected with their molecular weights and constituent monosaccharides [43]. Thence, impacts of different extraction procedures on molecular weights and constituent monosaccharides of LLPs were studied. As displayed in Fig. 1, the HPSEC-RID chromatograms of LLPs were significantly impacted by different extraction methods. Four polysaccharide fractions

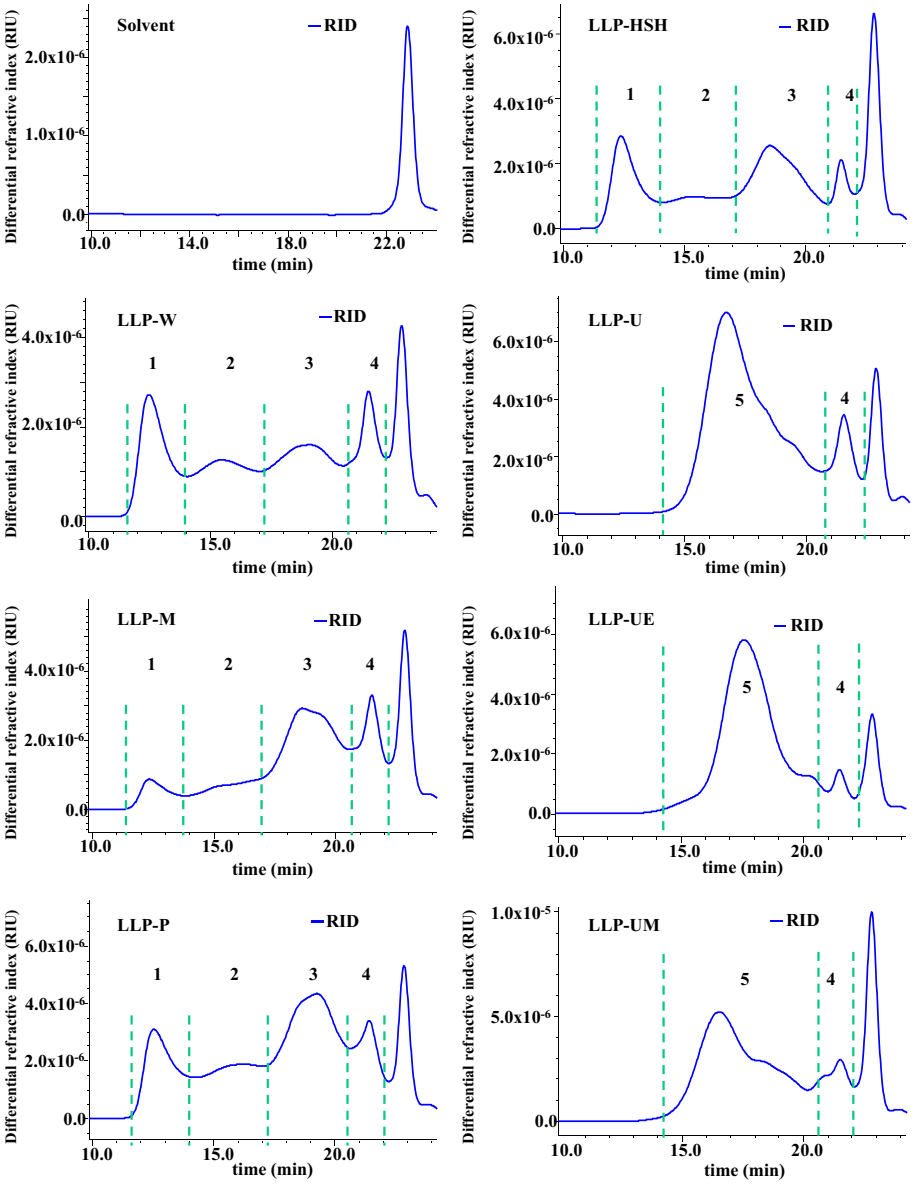

Fig. 1. High performance size exclusion chromatograms of LLPs. LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively.

(fractions 1 to 4) were measured in LLP-W, LLP-M, LLP-P, and LLP-HSH, while only two polysaccharide fractions (fraction 4 and fraction 5) were detected in LLP-U, LLP-UE, and LLP-UM. Molecular weights of polysaccharide fractions 1 to 4 in LLP-W, LLP-M, LLP-P, and LLP-HSH ranged from 9.178×10^6 to 9.971×10^6 Da, from 1.701×10^6 to 2.890×10^6 Da, from 0.216×10^6 to 0.450×10^6 Da, and from 0.106×10^6 to 0.366×10^6 Da (Table 2), respectively. Moreover, the polysaccharide fraction 5 in LLP-U, LLP-UE, LLP-UM might be related to the degradation of polysaccharide fraction 1 caused by ultrasonic processes [14,26], and the molecular weights of polysaccharide fraction 5 and polysaccharide fraction 4 in LLP-U, LLP-UE, and LLP-UM ranged from 0.531×10^6 to 3.373×10^6 Da, and from 0.099×10^6 to 1.366×10^6 Da, respectively. Furthermore, the significantly ($p < 0.05$) lower molecular weights were found in LLPs (LLP-U, LLP-UE, and LLP-UM) prepared by ultrasonic assisted processes among all tested samples. It has been confirmed that the high ultrasonic power might degrade the molecular weights of natural polysaccharides during extraction [26]. Results suggested that ultrasonic assisted processes could be used as an efficient technique for preparing more uniform polysaccharides. Furthermore, the polydispersities of polysaccharide fractions 1 to 4 in LLP-W, LLP-M, LLP-P, and LLP-HSH matched with the HPSEC chromatograms, and ranged from 1.07 to 1.29, from 1.15 to 1.39, from 1.07 to 1.23, and from 1.05 to 1.11, respectively. The polydispersities of polysaccharide fraction 5 and fraction 4 in LLP-U, LLP-UE, and LLP-UM ranged from 1.66 to 1.80, and from 1.07 to 1.12, respectively, which also matched with the HPSEC chromatograms.

Furthermore, Fig. 2A demonstrated that the HPLC-UV profiles of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE and LLP-UM were similar, and rhamnose (Rha), mannose (Man), galacturonic acid (GalA), arabinose (Ara), galactose (Gal), glucuronic acid (GlcA), glucose (Glc), and xylose (Xyl) were measured in LLPs. Result showed that the monosaccharide types of LLPs prepared by different extraction procedures were similar, which was in accordance with the reported study [12]. Table 2 summarized the molar ratios of Rha, Man, GalA, Ara, Gal, GlcA, Glc and Xyl in LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE and LLP-UM, which were varied by different extraction procedures. Similar studies have also demonstrated that different extraction methods can affect the molar ratios of monosaccharide compositions of natural polysaccharides [20,26]. Furthermore, the major monosaccharides in LLPs were determined as Rha, Man, GalA, Ara, and Gal, which suggested that rhamnogalacturonan I (RG I), homogalacturonan (HG), and mannan might exist in LLPs [12].

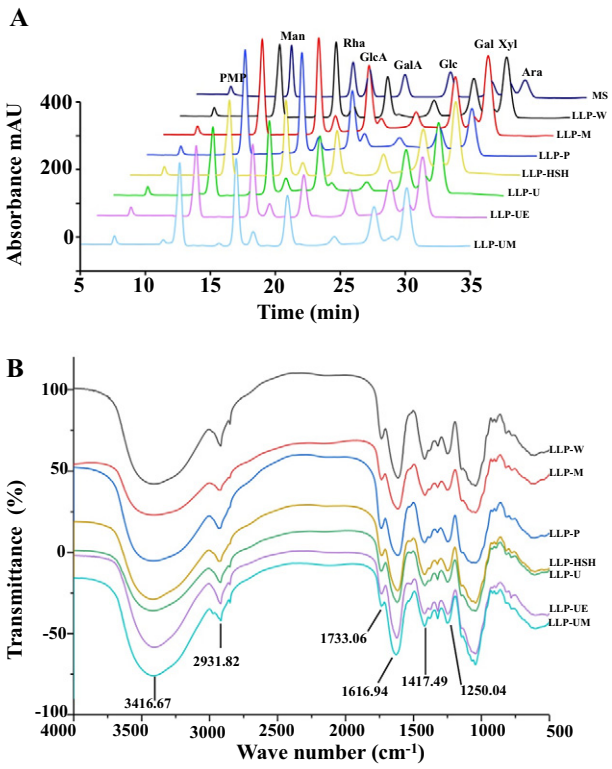

Fig. 2. High performance liquid chromatography profiles (A) and FT-IR spectra (B) of LLPs. PMP, 1-phenyl-3-methyl-5-pyrazolone; Rha, rhamnose; GalA, galacturonic acid; Ara, arabinose; Gal, galactose; GlcA, glucuronic acid; Glc, glucose; Xyl, xylose. LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively.

3.1.3. FT-IR spectra and esterification degree

The structural features of LLPs were also determined by the FT-IR spectroscopy. The FT-IR spectra of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-

Table 2
Molecular weight (M_w), polydispersity (M_w/M_n), and molar ratios of constituent monosaccharides of LLPs.

	LLP-W	LLP-M	LLP-P	LLP-HSH	LLP-U	LLP-UE	LLP-UM
$M_w \times 10^6$ (Da)							
Fraction 1	9.971 ± 0.228^a	9.799 ± 0.199^b	9.178 ± 0.199^d	9.332 ± 0.193^c	–	–	–
Fraction 2	1.701 ± 0.051^d	2.890 ± 0.063^a	2.246 ± 0.040^c	2.544 ± 0.108^b	–	–	–
Fraction 3	0.399 ± 0.018^a	0.216 ± 0.008^b	0.242 ± 0.008^b	0.450 ± 0.023^a	–	–	–
Fraction 4	0.190 ± 0.007^d	0.106 ± 0.004^f	0.153 ± 0.006^e	0.366 ± 0.016^c	0.099 ± 0.001^f	1.366 ± 0.006^a	0.826 ± 0.003^b
Fraction 5	–	–	–	–	0.531 ± 0.001^a	1.689 ± 0.003^c	3.373 ± 0.007^b
M_w/M_n							
Fraction 1	1.10	1.07	1.13	1.29	–	–	–
Fraction 2	1.21	1.39	1.33	1.15	–	–	–
Fraction 3	1.07	1.18	1.23	1.10	–	–	–
Fraction 4	1.11	1.07	1.05	1.09	1.12	1.08	1.07
Fraction 5	–	–	–	–	1.78	1.66	1.80
Monosaccharides and molar ratios							
Rhamnose	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mannose	0.84	0.83	0.78	0.82	0.82	0.90	0.84
Galacturonic acid	0.92	1.08	1.62	0.74	0.72	0.83	0.92
Arabinose	0.66	0.97	1.07	0.84	0.90	1.25	0.66
Galactose	0.37	0.70	0.70	0.59	0.61	0.84	0.37
Glucuronic acid	0.22	0.16	0.30	0.15	0.16	0.20	0.22
Glucose	0.15	0.59	0.15	0.12	0.51	0.41	0.15
Xylose	0.02	0.24	0.31	0.03	0.24	0.29	0.02

LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively.
Values represent mean ± standard deviation, and superscripts a–f differ significantly ($p < 0.05$) among LLPs.

U, LLP-UE, and LLP-UM were similar, which displayed in Fig. 2B. The strong absorption peaks at 3416.67 cm^{-1} and 2931.82 cm^{-1} were the characteristic stretching absorption peaks of O—H and C—H absorption [20,26]. The signal at 1733.06 cm^{-1} was the esterified carboxylic groups [12]. Furthermore, the signal at 1616.94 cm^{-1} was the C = O, demonstrating the LLPs were acidic polysaccharides [20,43]. The signal at 1417.49 cm^{-1} was related to C—H or O—H [44]. Further, the signal at 1250.04 cm^{-1} was the C—O—C, consisting with the existence of —OCH_3 [12]. The absorption peaks in 800–1200 cm^{-1} named fingerprint area were quiet similar, suggesting that the structures of LLPs prepared by different extraction methods were similar [45]. There is no signal at 1651 cm^{-1} and 1555 cm^{-1} which called typical protein band, indicating only a few proteins existed in LLPs. Additionally, the degrees of esterification (DE) of LLPs were also detected by FT-IR spectroscopy assay. As shown in Table 1, the highest DE value was detected in LLP-P (21.88%), followed by lower DE values in LLP-U (18.59%), LLP-UE (18.37%), LLP-HSH (18.15%), LLP-W (17.78%), and LLP-M (15.80%), and the lowest DE value in LLP-UM (12.80%). It has been confirmed that the low DE might be related to the harsh extraction conditions which promoted the de-esterification of polygalacturonic chains [46]. Several studies have demonstrated that the lower DE of natural polysaccharides may contribute to their higher antioxidant activity [26,38].

3.2. Rheological properties

The ideal functional properties of natural polysaccharides, such as foaming, gelling, thickening, and emulsifying properties, have been detected and discussed for a long time [47,48]. Therefore, impacts of different extraction procedures on the apparent viscosities of LLPs were studied. As shown in Fig. 3A, the apparent viscosities of LLPs associated with the shear rate. The non-Newtonian shear thinning behaviors could be found in LLPs solutions at low range of shear rate (0.01–50 s^{-1}), while the closely Newtonian flow behavior was found at high rang of shear rate (50–100 s^{-1}). It has been proved that the shear thinning behavior of polysaccharides could associate with the disentanglement of molecular chains in solution [49]. Furthermore, results showed that different extraction methods significantly affect the apparent viscosities of LLPs, which was similar with the previous study [23]. The strongest apparent viscosity was measured in LLP-W among all tested samples, while the lowest apparent viscosities were detected in LLPs (LLP-U, LLP-UE, and LLP-UM) prepared by ultrasonic assisted processes (Fig. 3A). It has been proved that the reduction of viscosity of natural polysaccharides is related to the decrease of molecular weight and its distribution [50], and LLPs (LLP-U, LLP-UE, and LLP-UM) prepared by ultrasonic assisted processes have relatively low molecular weights (Table 2).

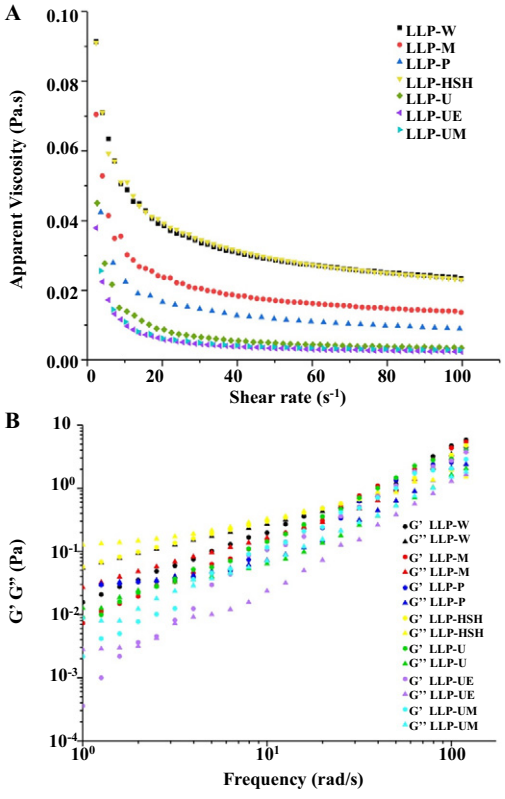

Fig. 3. Dependence of apparent viscosity on the shear rate (A) and plots of storage modulus G′ and loss modulus G″ against frequency (B) for LLPs. LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively.

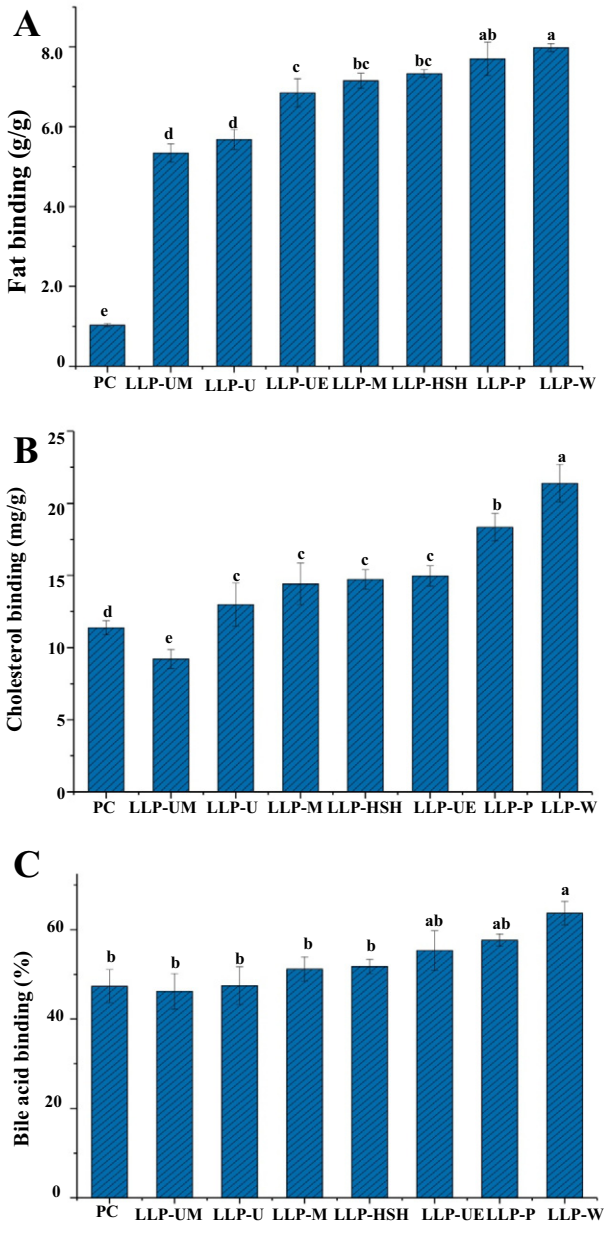

Fig. 4. The fat binding (A), cholesterol binding (B), and bile acid binding capacities (C) of LLPs. PC, positive control; LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively. Carboxymethyl cellulose was used as positive control in fat binding and cholesterol binding abilities, and cholestyramine was used as positive control in bile acid binding ability; values represent mean ± standard deviation, and superscripts a–e differ significantly ($p < 0.05$) among LLPs.

Polysaccharides are viscoelastic materials, the dynamic measurements can be taken to estimate the solid and liquid characters of LLPs [51]. The constant strain of 10% has been chosen for the frequency sweep, which belonged to the linear viscoelastic region. As shown in Fig. 3B, the storage modulus (G′) and loss modulus (G″) of LLPs solutions (0.4%, w/v) increased while the oscillation frequency increased at 25 °C. The liquid-like behavior of LLPs was found at low frequency where the G″ was higher than the G′. The weak gel-like behavior of LLPs was found at a certain frequency where the G′ began to exceed the G″ [37]. The higher molecular weight always displayed the higher G′ and G″ [52]. Moreover, the crossover points of G′ and G″ were significantly influenced by different extraction methods.

3.3. In vitro binding properties

Previous studies have shown that LLPs exhibited strong *in vitro* binding capacities [12]. Hence, impacts of different extraction procedures on the *in vitro* fat, cholesterol, and bile acid binding capacities of LLPs were studied. As shown in Fig. 4, the fat binding, cholesterol binding, and bile acid binding capacities of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE and LLP-UM ranged from 5.34 ± 0.23 g/g to 7.98 ± 0.10 g/g, from 9.21 ± 0.65 mg/g to 21.39 ± 1.30 mg/g, and from (46.21 ± 3.98)% to (63.70 ± 2.63)%, respectively. The higher binding properties can be easily found in LLPs when compared with the positive controls. Results demonstrated that different extraction methods significantly affected the fat binding, cholesterol binding, and bile acid binding capacities of LLPs. The stronger binding capacities were detected in both LLP-W and LLP-P among all tested samples, and the weakest binding capacities were found in LLP-UM among all tested samples. It has been proved that the strong binding capacities of natural polysaccharides are affected by their high molecular weights, high DEs, and wide molecular weight distributions [12,20,37,53]. The relatively strong binding capacities of LLP-P might be attributed to its high degree of esterification and molecular weights. Results suggested that the PWE could be used as an efficient extraction method for preparing LLPs with relatively high binding capacities for industrial applications.

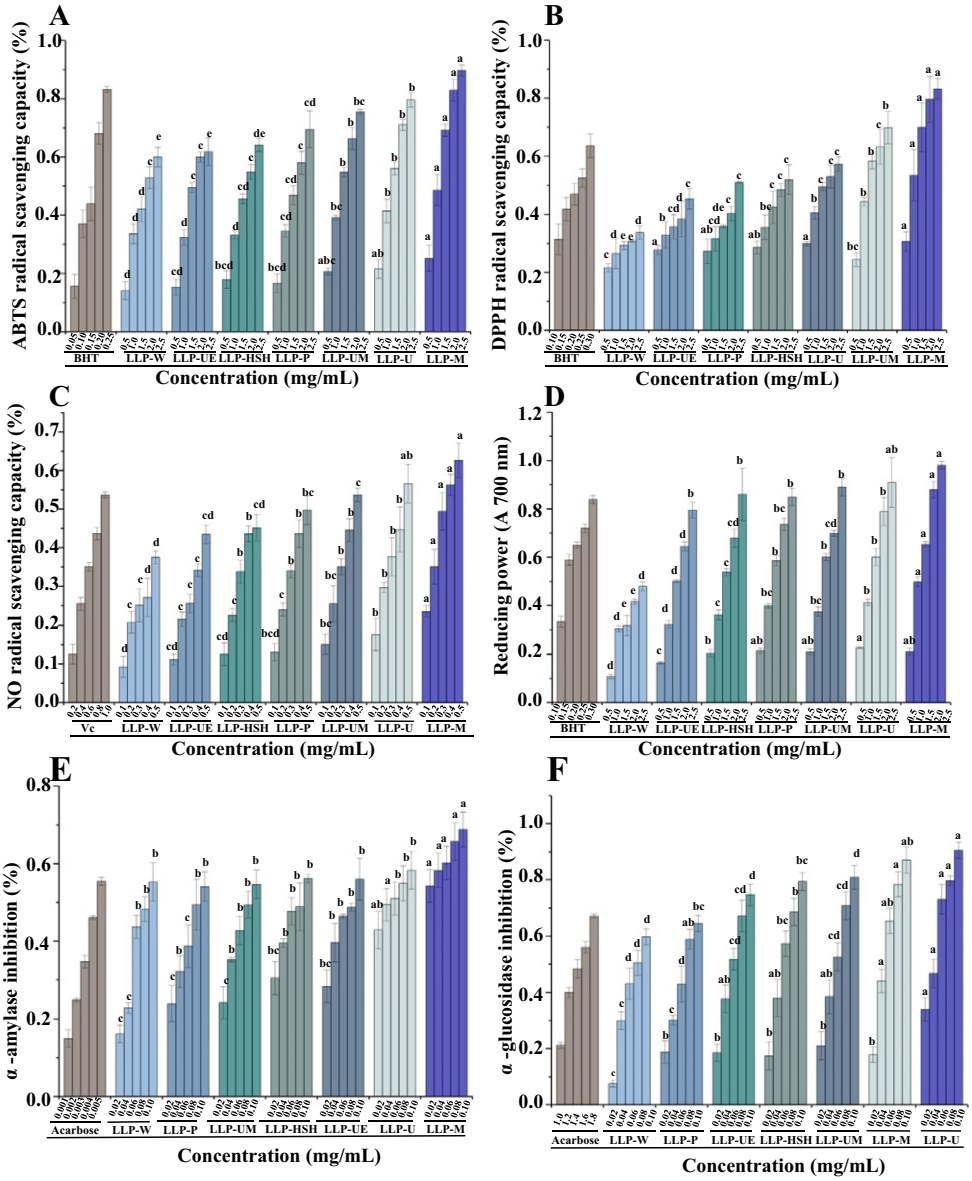

Fig. 5. ABTS (A), DPPH (B), and nitric oxide (C) radical scavenging activities, and reducing powers (D), as well as inhibitory effects on α-amylase (E) and α-glucosidase (F) of LLPs. LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively. The error bars are standard deviations; significant ($p < 0.05$) differences are shown by data bearing different letters (a–e).

3.4. In vitro antioxidant activities

Extracts of loquat leaves have been proved to possess strong antioxidant activities [11]. Therefore, impacts of different extraction procedures on the antioxidant activities of LLPs were studied. Fig. 5A, B, C, and D showed the ABTS, DPPH, and nitric oxide radical scavenging activities, and reducing powers of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM. As displayed in Fig. 5A, B, and C, the IC_{50} values of ABTS, DPPH, and nitric oxide radical scavenging activities of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were determined from 0.942 to 1.831 mg/mL, from 0.871 to 6.50 mg/mL, and from 0.317 to 0.916 mg/mL, respectively. Furthermore, as shown in Fig. 5D, LLPs also displayed strong reducing powers, and the absorbance at 700 nm of LLPs ranged from 0.479 to 1.012 at the concentration of 2.5 mg/mL. Results indicated that all tested samples exhibited moderate antioxidant activities when compared with the positive controls. However, the antioxidant activities of LLPs were higher than that of pectic-polysaccharides extracted from other resources such as okra and kiwifruit [37,44]. Furthermore, results demonstrated that different extraction procedures significantly influenced antioxidant activities of LLPs. The higher antioxidant activities were found in LLP-M and LLP-U among all tested samples, and the lowest antioxidant activities were detected in LLP-W among all tested samples. Generally, the antioxidant activities of natural polysaccharides may be related to their high contents of total phenolics and uronic acids, low esterification degrees, and low molecular weights [36,54]. The high content of total phenolics and low

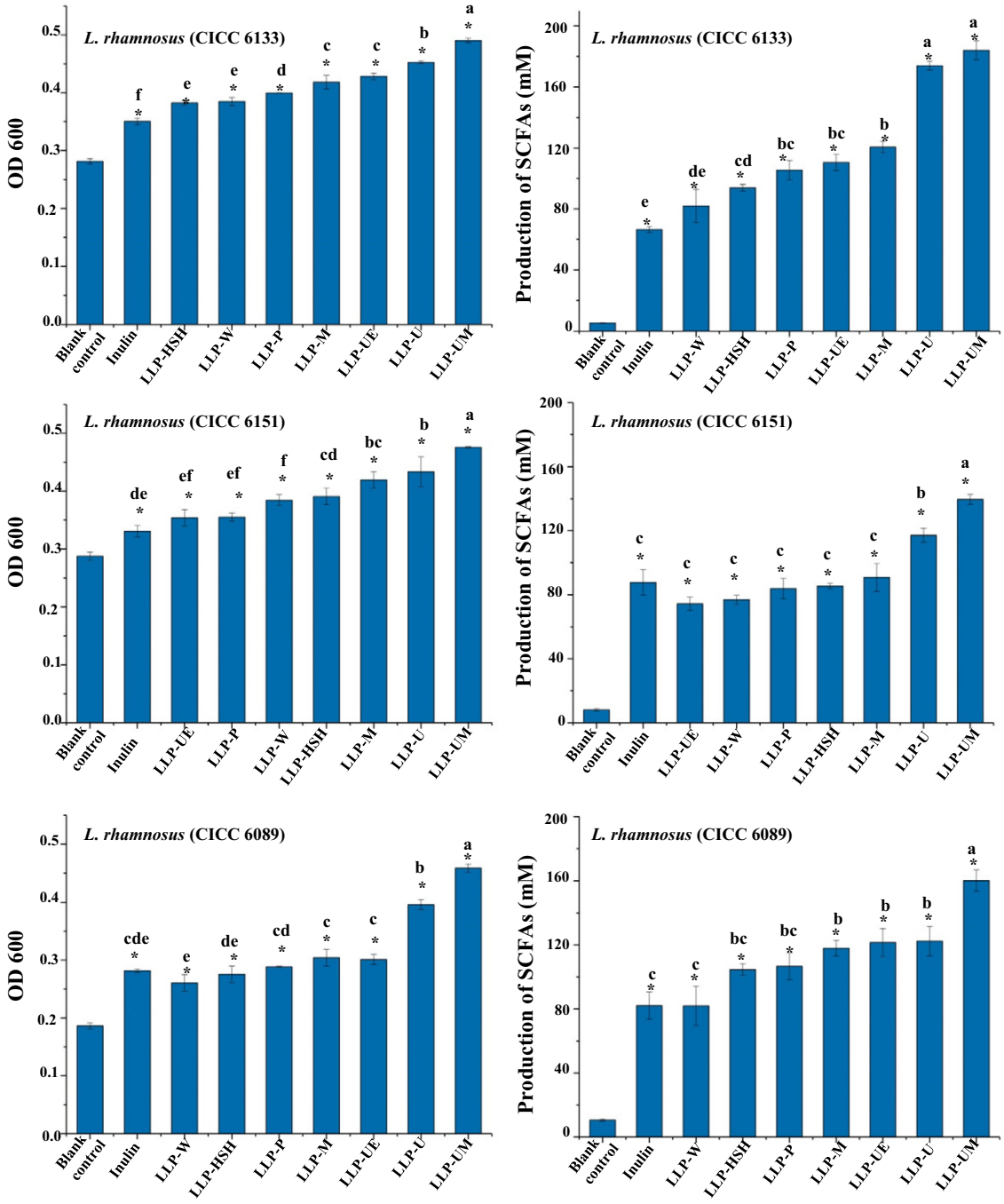

Fig. 6. Effects of LLPs on the growth (left) and production of total SCFAs (right) of three *Lactobacilli* strains. LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM, polysaccharides extracted by HWE, MAE, PWE, HSHE, UAE, UAEE, and UMAE, respectively. Values represent mean ± standard deviation; Significant ($p < 0.05$) differences between blank control and samples are shown by *; Superscripts a-f differ significantly ($p < 0.05$) among LLPs and inulin.

molecular weights might contribute to the strong antioxidant activities of LLP-M and LLP-U, respectively. Results indicated that the MAE and UAE could be the potential extraction techniques for obtaining LLPs with high antioxidant effects.

3.5. In vitro α-amylase and α-glucosidase inhibitory effects

The major strategy to counteract metabolic alterations associated with type 2 diabetes is the inhibition of α-glucosidase and α-amylase activities. Previous study has demonstrated that pectic-polysaccharides extracted from loquat leaves exhibit outstanding *in vitro* α-amylase and α-glucosidase inhibitory effects [12]. Therefore, impacts of different extraction procedures on the *in vitro* α-amylase and α-glucosidase inhibitory effects of LLPs were studied. As shown in Fig. 5E and F, the excellent inhibition effects on α-amylase and α-glucosidase of LLPs were detected. The IC_{50} values of α-amylase inhibition of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, LLP-UE, and LLP-UM were determined from 0.015 to 0.088 mg/mL. Results indicated that LLPs exerted moderate inhibitory effects on α-amylase when compared with the positive control (acarbose). Different extraction methods significantly affected the inhibitory effects of LLPs on α-amylase. The higher α-amylase inhibitory effects were measured in LLP-M and LLP-U among all tested samples. Furthermore, the IC_{50} values of α-glucosidase inhibition of LLP-W, LLP-M, LLP-P, LLP-HSH, LLP-U, and LLP-UE were determined from 0.031 to 0.234 mg/mL. Compared with the positive control (acarbose, IC_{50} = 1.441 mg/mL), LLPs prepared by different extraction methods displayed strong inhibitory effects on α-glucosidase. The α-glucosidase inhibitory effects of LLPs were also affected by different extraction processes. The higher α-glucosidase inhibitory effects were also detected in LLP-M and LLP-U among all tested samples. The strong *in vitro* α-amylase and α-glucosidase inhibitory effects of LLP-U and LLP-M might be related to their high contents of uronic acids and high contents of total phenolics [12,55–57]. Results suggested that both UAE and MAE could be potential extraction methods with high efficiency for preparing LLPs with high inhibitory effects on α-amylase and α-glucosidase.

3.6. In vitro prebiotic activities

It has been proved that beneficial effects of natural polysaccharides are greatly associated with the effects on gut microbiota [39]. Hence, the effects of LLPs prepared by different extraction methods on the growth of probiotics were investigated. As shown in Fig. 6, all LLPs and inulin (positive control) could significantly promote the growth of three *Lactobacilli* strains, including *L. rhamnosus* (CICC 6133), *L. rhamnosus* (CICC 6151), and *L. acidophilus* (CICC 6089). Compared with the inulin, all LLPs exhibited strong prebiotic effects. Furthermore, the prebiotic effects of LLPs were also affected by different extraction processes, which were similar with the previous study that prebiotic activities of natural polysaccharides were significantly influenced by different extraction processes [39]. The higher promotion effects on the growth of three *Lactobacilli* strains were determined in LLP-UM and LLP-U among all tested samples. It has been confirmed that the low molecular weights and low viscosities of natural polysaccharides may contribute to their high prebiotic effects [34,39,40]. Therefore, the high prebiotic effects of LLP-UM and LLP-U might be related to their relatively low viscosities and low molecular weights. Furthermore, as displayed in Fig. 6, all LLPs and inulin could significantly promote the production of total SCFAs from *Lactobacilli* strains. Compare with the inulin, all LLPs also exhibited strong abilities to promote the production of total SCFAs from *Lactobacilli* strains. The higher contents of total SCFAs produced by *Lactobacilli* strains were also observed in LLP-UM and LLP-U. Results revealed that the prebiotic activities of LLPs were significantly impacted by different extraction methods. Both UAE and UMAE methods could be potential techniques for preparing LLPs with desired prebiotic effects for applications in the pharmaceutical and functional food industries.

4. Conclusions

Physicochemical characteristics and bioactivities of LLPs were noticeably affected by different extraction methods. Degrees of esterification, contents of phenolics, molar ratios of constituent monosaccharides, apparent viscosities, and molecular weights of LLPs were varied by different extraction methods. The strong binding capacities were measured in LLP-W and LLP-P. The strong antioxidant activities and inhibitory effects on α-amylase and α-glucosidase were found in LLP-M and LLP-U. Furthermore, the strong prebiotic effects were detected in LLP-U and LLP-UM. These findings could provide scientific foundations for selecting appropriate extraction methods to obtain LLPs with desired bioactivities for industrial applications.

CRediT authorship contribution statement

Yuan Fu: Data curation, Formal analysis, Investigation, Resources, Software, Writing - original draft. **Fen Li:** Formal analysis, Investigation, Validation, Resources, Software, Writing - original draft. **Ye Ding:** Formal analysis, Investigation. **Hua-Yu Li:** Formal analysis, Investigation. **Xian-Rong Xiang:** Formal analysis, Investigation. **Qiong Ye:** Formal analysis, Investigation. **Jian Zhang:** Formal analysis, Investigation. **Li Zhao:** Formal analysis, Investigation, Software. **Wen Qin:** Resources, Software. **Ren-You Gan:** Formal analysis, Funding acquisition, Methodology, Supervision. **Ding-Tao Wu:** Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Project administration, Writing - review & editing.

Acknowledgments

This work was supported by the Scientific Research Foundation of Sichuan Agricultural University (grant number 03120321), the National Natural Science Foundation of China (grant number 31901690), the Scientific Research Fund Project of Department of Science and Technology of Sichuan Province (grant number 2018JY0149), and the Technology Innovation Program of Chinese Academy of Agricultural Sciences (grant number ASTIP).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

References

[1] W. Li, X. Wang, J. Zhang, X. Zhao, Y. Wu, S. Tan, Q. Zheng, X. Gao, Multivariate analysis illuminates the effects of vacuum drying on the extractable and nonextractable polyphenols profile of loquat fruit, J. Food Sci. 84 (4) (2019) 726–737.
[2] K. Tanaka, S. Tamaru, S. Nishizono, Y. Miyata, K. Tamaya, T. Matsui, T. Tanaka, Y. Echizen, I. Ikeda, Hypotriacylglycerolemic and antiobesity properties of a new fermented tea product obtained by tea-rolling processing of third-crop green tea (*Camellia sinensis*) leaves and loquat (*Eriobotrya japonica*) leaves, Biosci. Biotechnol. Biochem. 74 (8) (2010) 1606–1612.
[3] P.P. Zar, S. Yano, K. Sakao, F. Hashimoto, T. Nakano, M. Fujii, D.X. Hou, *In vitro* anticancer activity of loquat tea by inducing apoptosis in human leukemia cells, Biosci. Biotechnol. Biochem. 78 (10) (2014) 1731–1737.
[4] T. Shii, T. Tanaka, S. Watarumi, Y. Matsuo, Y. Miyata, K. Tamaya, S. Tamaru, K. Tanaka, T. Matsui, I. Kouno, Polyphenol composition of a functional fermented tea obtained by tea-rolling processing of green tea and loquat leaves, J. Agric. Food Chem. 59 (13) (2011) 7253–7260.
[5] Y. Liu, W. Zhang, C. Xu, X. Li, Biological activities of extracts from loquat (*Eriobotrya japonica* Lindl.): a review, Int. J. Mol. Sci. 17 (12) (2016) 1983.
[6] Y.X. Wu, T.Y. Jian, H. Lv, X.Q. Ding, Y.Y. Zuo, B.R. Ren, J. Chen, W.L. Li, Antitussive and expectorant properties of growing and fallen leaves of loquat (*Eriobotrya japonica*), Rev. Bras 28 (2) (2018) 239–242.
[7] D.S. Cha, J.S. Eun, H. Jeon, Anti-inflammatory and antinociceptive properties of the leaves of *Eriobotrya japonica*, J. Ethnopharmacol. 134 (2) (2011) 305–312.
[8] T. Jian, X. Ding, Y. Wu, B. Ren, W. Li, H. Lv, J. Chen, Hepatoprotective effect of loquat leaf flavonoids in PM2.5-induced non-alcoholic fatty liver disease via regulation of IRs-1/Akt and CYP2E1/JNK pathways, Int. J. Mol. Sci. 19 (10) (2018) 3005.

[9] T. Jian, X. Ao, Y. Wu, H. Lv, L. Ma, L. Zhao, B. Tong, B. Ren, J. Chen, W. Li, Total sesquiterpene glycosides from Loquat (*Eriobotrya japonica*) leaf alleviate high-fat diet induced non-alcoholic fatty liver disease through cytochrome P450 2E1 inhibition, Biomed. Pharmacother. 91 (2017) 229–237.
[10] C.C. Shih, C.H. Lin, J.B. Wu, *Eriobotrya japonica* improves hyperlipidemia and reverses insulin resistance in high-fat-fed mice, Phytother. Res. 24 (12) (2010) 1769–1780.
[11] Y. Hong, S. Lin, Y. Jiang, M. Ashraf, Variation in contents of total phenolics and flavonoids and antioxidant activities in the leaves of 11 *Eriobotrya* species, Plant Foods Hum. Nutr. 63 (4) (2008) 200–204.
[12] Y. Fu, Q. Yuan, S. Lin, W. Liu, G. Du, L. Zhao, Q. Zhang, D.R. Lin, Y.T. Liu, W. Qin, D.Q. Li, D.T. Wu, Physicochemical characteristics and biological activities of polysaccharides from the leaves of different loquat (*Eriobotrya japonica*) cultivars, Int. J. Biol. Macromol. 135 (2019) 274–281.
[13] S. Chen, H. Shang, J. Yang, R. Li, H. Wu, Effects of different extraction techniques on physicochemical properties and activities of polysaccharides from comfrey (*Symphytum officinale* L.) root, Ind. Crop. Prod. 121 (2018) 18–25.
[14] L. He, X. Yan, J. Liang, S. Li, H. He, Q. Xiong, X. Lai, S. Hou, S. Huang, Comparison of different extraction methods for polysaccharides from *Dendrobium officinale* stem, Carbohydr. Polym. 198 (2018) 101–108.
[15] S. Yang, Y. Li, D. Jia, K. Yao, W. Liu, The synergy of Box-Behnken designs on the optimization of polysaccharide extraction from mulberry leaves, Ind. Crop. Prod. 99 (2017) 70–78.
[16] G. Chen, Q. Yuan, M. Saeeduddin, S. Ou, X. Zeng, H. Ye, Recent advances in tea polysaccharides: extraction, purification, physicochemical characterization and bioactivities, Carbohydr. Polym. 153 (2016) 663–678.
[17] S. He, X. Wang, Y. Zhang, J. Wang, H. Sun, J. Wang, X. Cao, Y. Ye, Isolation and prebiotic activity of water-soluble polysaccharides fractions from the bamboo shoots (*Phyllostachys praecox*), Carbohydr. Polym. 151 (2016) 295–304.
[18] C. Chen, B. Zhang, Q. Huang, X. Fu, R.H. Liu, Microwave-assisted extraction of polysaccharides from *Moringa oleifera* Lam. leaves: characterization and hypoglycemic activity, Ind. Crop. Prod. 100 (2017) 1–11.
[19] G. Chen, C. Fang, C. Ran, Y. Tan, Q. Yu, J. Kan, Comparison of different extraction methods for polysaccharides from bamboo shoots (*Chimonobambusa quadrangularis*) processing by-products, Int. J. Biol. Macromol. 130 (2019) 903–914.
[20] Q. Yuan, S. Lin, Y. Fu, X.R. Nie, W. Liu, Y. Su, Q.H. Han, L. Zhao, Q. Zhang, D.R. Lin, W. Qin, D.T. Wu, Effects of extraction methods on the physicochemical characteristics and biological activities of polysaccharides from okra (*Abelmoschus esculentus*), Int. J. Biol. Macromol. 127 (2019) 178–186.
[21] Y.J. Cho, A.T. Getachew, P.S. Saravana, B.S. Chun, Optimization and characterization of polysaccharides extraction from Giant African snail (*Achatina fulica*) using pressurized hot water extraction (PHWE), Bioact. Carbohydr. Diet. Fibre 18 (2019), 100179.
[22] X. Guo, W. Zhao, X. Pang, X. Liao, X. Hu, J. Wu, Emulsion stabilizing properties of pectins extracted by high hydrostatic pressure, high-speed shearing homogenization and traditional thermal methods: a comparative study, Food Hydrocoll. 35 (2014) 217–225.
[23] X. Guo, W. Zhao, X. Liao, X. Hu, J. Wu, X. Wang, Extraction of pectin from the peels of pomelo by high-speed shearing homogenization and its characteristics, LWT Food Sci. Technol. 79 (2017) 640–646.
[24] J. Zhao, W. Zhang, X. Zhang, X. Zhang, C. Lu, Y. Deng, Extraction of cellulose nanofibrils from dry softwood pulp using high shear homogenization, Carbohydr. Polym. 97 (2) (2013) 695–702.
[25] T. Lin, Y. Liu, C. Lai, T. Yang, J. Xie, Y. Zhang, The effect of ultrasound assisted extraction on structural composition, antioxidant activity and immunoregulation of polysaccharides from *Ziziphus jujuba* Mill var. spinosa seeds, Ind. Crop. Prod. 125 (2018) 150–159.
[26] H. Guo, Q. Yuan, Y. Fu, W. Liu, Y.H. Su, H. Liu, C.Y. Wu, L. Zhao, Q. Zhang, D.R. Lin, H. Chen, W. Qin, D.T. Wu, Extraction optimization and effects of extraction methods on the chemical structures and antioxidant activities of polysaccharides from snow chrysanthemum (*Coreopsis tinctoria*), Polymers 11 (2) (2019) 215.
[27] J.B. Pu, B.H. Xia, Y.J. Hu, H.J. Zhang, J. Chen, J. Zhou, W.Q. Liang, P. Xu, Multi-optimization of ultrasonic-assisted enzymatic extraction of atratylodes macrocephala polysaccharides and antioxidants using response surface methodology and desirability function approach, Molecules 20 (12) (2015) 22220–22235.
[28] S. Qian, X. Fang, D. Dan, E. Diao, Z. Lu, Ultrasonic-assisted enzymatic extraction of a water soluble polysaccharide from dragon fruit peel and its antioxidant activity, RSC Adv. 8 (73) (2018) 42145–42152.
[29] L. Li, X. Li, C. Ding, S. Yuan, Z. Zhang, Y. Chen, C. Hu, M. Yuan, Ultrasonic-assisted enzymatic extraction and antioxidant activity of polysaccharides from *Setaria viridis*, Sep. Sci. Technol. 51 (11) (2016) 1798–1805.
[30] A. Masci, S. Carradori, M.A. Casadei, P. Paolicelli, S. Petralito, R. Ragno, S. Cesa, *Lycium barbarum* polysaccharides: extraction, purification, structural characterisation and evidence about hypoglycaemic and hypolipidaemic effects. A review, Food Chem. 254 (2018) 377–389.
[31] H. Sun, C. Li, Y. Ni, L. Yao, H. Jiang, X. Ren, Y. Fu, C. Zhao, Ultrasonic/microwave-assisted extraction of polysaccharides from camptotheca acuminata fruits and its antitumor activity, Carbohydr. Polym. 206 (2019) 557–564.
[32] M.A. Praveen, K.R.K. Parvathy, P. Balasubramanian, R. Jayabalan, An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota, Trends Food Sci. Technol. 92 (2019) 46–64.
[33] J.K. Yan, L.X. Wu, W.D. Cai, G.S. Xiao, Y. Duan, H. Zhang, Subcritical water extraction-based methods affect the physicochemical and functional properties of soluble dietary fibers from wheat bran, Food Chem. 298 (2019), 124987.
[34] X. Wang, M. Huang, F. Yang, H. Sun, X. Zhou, Y. Guo, X. Wang, M. Zhang, Rapeseed polysaccharides as prebiotics on growth and acidifying activity of probiotics *in vitro*, Carbohydr. Polym. 125 (2015) 232–240.
[35] L. Wang, T. Li, F. Liu, D. Liu, Y. Xu, Y. Yang, Y. Zhao, H. Wei, Ultrasonic-assisted enzymatic extraction and characterization of polysaccharides from dandelion (*Taraxacum officinale*) leaves, Int. J. Biol. Macromol. 126 (2019) 846–856.
[36] D.D. Shen, X. Li, Y.L. Qin, M.T. Li, Q.H. Han, J. Zhou, S. Lin, L. Zhao, Q. Zhang, W. Qin, D.T. Wu, Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages, J. Food Sci. Technol. 56 (3) (2019) 1275–1286.
[37] X.R. Nie, H.Y. Li, G. Du, S. Lin, R. Hu, H.Y. Li, L. Zhao, Q. Zhang, H. Chen, D.T. Wu, W. Qin, Structural characteristics, rheological properties, and biological activities of polysaccharides from different cultivars of okra (*Abelmoschus esculentus*) collected in China, Int. J. Biol. Macromol. 139 (2019) 459–467.
[38] Q.H. Han, W. Liu, H.Y. Li, J.L. He, H. Guo, S. Lin, L. Zhao, H. Chen, Y.W. Liu, D.T. Wu, S.Q. Li, W. Qin, Extraction optimization, physicochemical characteristics, and antioxidant activities of polysaccharides from kiwifruit (*Actinidia chinensis* Planch.), Molecules 24 (3) (2019) 461.
[39] F. Huang, H. Liu, R. Zhang, L. Dong, L. Liu, Y. Ma, X. Jia, G. Wang, M. Zhang, Physicochemical properties and prebiotic activities of polysaccharides from longan pulp based on different extraction techniques, Carbohydr. Polym. 206 (2019) 344–351.
[40] G. Chen, X. Chen, B. Yang, Q. Yu, X. Wei, Y. Ding, J. Kan, New insight into bamboo shoot (*Chimonobambusa quadrangularis*) polysaccharides: impact of extraction processes on its prebiotic activity, Food Hydrocoll. 95 (2019) 367–377.
[41] Y. Xu, F. Cai, Z. Yu, L. Zhang, X. Li, Y. Yang, G. Liu, Optimisation of pressurised water extraction of polysaccharides from blackcurrant and its antioxidant activity, Food Chem. 194 (2016) 650–658.
[42] Q. Guo, Q. Ma, Z. Xue, X. Gao, H. Chen, Studies on the binding characteristics of three polysaccharides with different molecular weight and flavonoids from corn silk (*Maydis stigma*), Carbohydr. Polym. 198 (2018) 581–588.
[43] D.T. Wu, H. Guo, S. Lin, S.C. Lam, L. Zhao, D.R. Lin, W. Qin, Review of the structural characterization, quality evaluation, and industrial application of *Lycium barbarum* polysaccharides, Trends Food Sci. Technol. 79 (2018) 171–183.
[44] D.T. Wu, W. Liu, Q.H. Han, G. Du, H.Y. Li, Q. Yuan, Y. Fu, L. Zhao, Q. Zhang, S.Q. Li, W. Qin, Physicochemical characteristics and antioxidant activities of non-starch polysaccharides from different kiwifruits, Int. J. Biol. Macromol. 136 (2019) 891–900.
[45] H. Gao, J.J. Wen, J.L. Hu, Q.X. Nie, H.H. Chen, T. Xiong, S.P. Nie, M.Y. Xie, Polysaccharide from fermented *Momordica charantia* L. with *Lactobacillus plantarum* NCU116 ameliorates type 2 diabetes in rats, Carbohydr. Polym. 201 (2018) 624–633.
[46] D.L. Su, P.J. Li, S.Y. Quek, Z.Q. Huang, Y.J. Yuan, G.Y. Li, Y. Shan, Efficient extraction and characterization of pectin from orange peel by a combined surfactant and microwave assisted process, Food Chem. 286 (2019) 1–7.
[47] Q. Jin, Z. Cai, X. Li, M.P. Yadav, H. Zhang, Comparative viscoelasticity studies: corn fiber gum versus commercial polysaccharide emulsifiers in bulk and at air/liquid interfaces, Food Hydrocoll. 64 (2017) 85–98.
[48] J. Gao, T. Zhang, Z.Y. Jin, X.M. Xu, J.H. Wang, X.Q. Zha, H.Q. Chen, Structural characterisation, physicochemical properties and antioxidant activity of polysaccharide from *Lilium lancifolium* Thunb, Food Chem. 169 (2015) 430–438.
[49] Y. Xu, N. Liu, X. Fu, L. Wang, Y. Yang, Y. Ren, J. Liu, L. Wang, Structural characteristics, biological, rheological and thermal properties of the polysaccharide and the degraded polysaccharide from raspberry fruits, Int. J. Biol. Macromol. 132 (2019) 109–118.
[50] W. Wang, X. Ma, P. Jiang, L. Hu, Z. Zhi, J. Chen, T. Ding, X. Ye, D. Liu, Characterization of pectin from grapefruit peel: a comparison of ultrasound-assisted and conventional heating extractions, Food Hydrocoll. 61 (2016) 730–739.
[51] L.M. Nwokocha, P.A. Williams, Rheological characterization of the galactomannan from *Leucaena leucocephala* seed, Carbohydr. Polym. 90 (2) (2012) 833–838.
[52] L. Wang, B. Zhang, J. Xiao, Q. Huang, C. Li, X. Fu, Physicochemical, functional, and biological properties of water-soluble polysaccharides from *Rosa roxburghii* Tratt fruit, Food Chem. 249 (2018) 127–135.
[53] S.H. Lee, G.Y. Jang, M.Y. Kim, I.G. Hwang, H.Y. Kim, K.S. Woo, M.J. Lee, T.J. Kim, J. Lee, H.S. Jeong, Physicochemical and in vitro binding properties of barley beta-glucan treated with hydrogen peroxide, Food Chem. 192 (2016) 729–735.
[54] J. Sheng, Y. Sun, Antioxidant properties of different molecular weight polysaccharides from *Athyrium multidentatum* (Doll.) Ching, Carbohydr. Polym. 108 (2014) 41–45.
[55] J.K. Yan, L.X. Wu, Z.R. Qiao, W.D. Cai, H. Ma, Effect of different drying methods on the product quality and bioactive polysaccharides of bitter gourd (*Momordica charantia* L.) slices, Food Chem. 271 (2019) 588–596.
[56] M. Espinal-Ruiz, F. Parada-Alfonso, L.P. Restrepo-Sánchez, C.E. Narváez-Cuenca, Inhibition of digestive enzyme activities by pectic polysaccharides in model solutions, Bioact. Carbohydr. Diet. Fibre 4 (1) (2014) 27–38.
[57] H.Y. Li, Q. Yuan, Y.L. Yang, Q.H. Han, J.L. He, L. Zhao, Q. Zhang, S.X. Liu, D.R. Lin, D.T. Wu, W. Qin, Phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of different kiwifruits, Molecules 23 (11) (2018) 2953.