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Comparison of structural characteristics and bioactivities of polysaccharides from loquat leaves prepared by different drying techniques

Yuan Fu [a,1], Kang-Lin Feng [a,1], Si-Yu Wei [a], Xian-Rong Xiang [a], Ye Ding [a], Hua-Yu Li [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

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ABSTRACT

In the present study, freeze drying, hot-air drying, vacuum drying, and microwave drying at the microwave powers of 400, 600, and 800 W, respectively, were utilized to dry loquat leaves for evaluating the effects of different drying techniques on the physicochemical structures and bioactivities of polysaccharides extracted from loquat leaves (LLPs). Results demonstrated that the physicochemical structures and bioactivities of LLPs significantly affected by different drying techniques. The degrees of esterification, molar ratios of constituent monosaccharides, contents of uronic acids, apparent viscosities, and molecular weights of LLPs were varied by different drying techniques. Additionally, LLPs, particularly LLP-M4 which extracted from loquat leaves prepared by microwave drying at the power of 400 W, exerted remarkable *in vitro* binding capacities, strong inhibitory effects on α-amylase and α-glucosidase, and obvious antioxidant activities. Results indicated that the microwave drying could be an efficient drying technique before extraction of bioactive LLPs, and LLPs had great potential applications in the functional food and pharmaceutical 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 tea material and a famous traditional Chinese medicine [2,3]. Generally, loquat leaves are widely utilized for the treatment of different diseases, such as diabetes, chronic bronchitis, asthma, pain, cough, and inflammation [3]. Extracts of loquat leaves have been proved to possess various bioactivities [4], such as anti-diabetic, anti-obesity [5], anti-inflammatory [6] antioxidant [7], and hepatoprotective effects [8]. Moreover, previous studies have demonstrated that polysaccharides which richly distribute in loquat leaf (ranged 3.62% to 5.29%) have obvious *in vitro* anti-diabetic and *in vitro* anti-obesity effects [9]. Hence, polysaccharides extracted from loquat leaves (LLPs) have potential applications in the functional food and pharmaceutical industries.

The fresh loquat leaf usually contains >80% of water, and is susceptible to exacerbation, resulting in nutrient loss [10]. Therefore, an appropriate drying technique is pursued for fresh loquat leaves to decrease nutrient loss and prolong shelf-life. Generally, the loquat leaves are widely used to prepare traditional Chinese medicine and tea materials after drying process [2]. Drying process plays an important role in the development of desirable loquat leaf products. However, the conventional drying procedures, such as freeze drying (FD), hot-air drying (HD), and vacuum drying (VD), are always associated with long drying time, high energy consumption, and low efficiency [11]. Therefore, developing a highly efficient drying technique for drying loquat leaves is essential. Microwave drying (MD) is widely used as an efficient drying

Abbreviations: FD, freeze drying; HD, hot-air drying; VD, vacuum drying; MD, microwave drying; MD-400, microwave drying at the microwave power of 400 W; MD-600, microwave drying at the microwave power of 600 W; MD-800, microwave drying at the microwave power of 800 W; LLPs, polysaccharides extracted from loquat leaves; LLP-F, polysaccharides extracted from loquat leaves dried by freeze drying; LLP-H, polysaccharides extracted from loquat leaves dried by hot-air drying; LLP-V, polysaccharides extracted from loquat leaves dried by vacuum drying; LLP-M4, polysaccharides extracted from loquat leaves dried by microwave drying at 400 W; LLP-M6, polysaccharides extracted from loquat leaves dried by microwave drying at 600 W; LLP-M8, polysaccharides extracted from loquat leaves dried by microwave drying at 800 W.

* 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.

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technique for drying fresh fruits and vegetables [12]. MD has various advantages over conventional drying techniques such as a higher drying rate, better process control, and homogeneous energy delivery on the material [13]. It has also been confirmed that MD can maintain the color, a faint aroma, and antioxidant property of herbal medicine, and the shorter drying time of MD also leads to better nutrient preservation [10,14]. Meanwhile, several studies have suggested that the physicochemical properties and bioactivities of crude polysaccharides from the fruits, vegetables, and medicinal plants extremely influenced by different drying techniques [15,16]. However, whether the physicochemical structures and bioactivities of LLPs are also affected by different drying techniques is still unknown. As is known to all, there are limited investigations about effects of different drying techniques on the physicochemical structures and bioactivities of LLPs.

Therefore, for the purpose of evaluating effects of different drying techniques on the physicochemical structures and bioactivities of LLPs, four drying techniques, including FD, HD, VD, and MD at 400, 600, and 800 W, respectively, were applied to dry loquat leaves, and then the chemical structures, rheological properties, *in vitro* antioxidant, anti-obesity, and anti-diabetic effects of LLPs prepared by different drying techniques were investigated and compared.

2. Materials and methods

2.1. Material and reagents

Loquat leaves (*Eriobotrya japonica* cv. *Chuannong*8) were purchased from Chengdu, Sichuan Province, China. α-Glucosidase (10 U/mg), soluble starch, α-amylase (1000 U/mg), 4-nitrophenyl β-D-glucopyranoside (pNPG), acarbose, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), vitamin C, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), xylose (Xyl), rhamnose (Rha), galacturonic acid (GalA), mannose (Man), glucuronic acid (GlcA), galactose (Gal), glucose (Glc), and arabinose (Ara) were bought from Sigma-Aldrich (St. Louis, MO, USA). A free cholesterol assay kit and heat stable α-amylase (40 U/mg) were purchased from Solarbio (Beijing, China).

2.2. Drying experiments

Before drying, loquat leaves were cut into square pieces of about 4 cm × 4 cm. Four drying techniques, including FD, HD, and VD, as well as MD at the microwave powers of 400 W (MD-400), 600 W (MD-600), and 800 W (MD-800), respectively, were carried out. In brief, for the FD process, loquat leaves were freeze-dried at −40 °C for 48 h (SCIENTZ-12N, Ningbo Scientz Biotechnology Co., Ltd., China). For the HD process, loquat leaves were dried at 75 °C for 2 h (101A-3, Shanghai Experimental Instrument Factory Co., Ltd., China) [15]. For the VD process, loquat leaves were dried at 50 °C for 14.5 h (DZF-6050, Shanghai San Fa Scientific Instruments Factory Co., Ltd., China) [17]. MD experiments were carried out at the microwave powers of 400 W, 600 W, and 800 W for 4.5 min, 3.0 min, and 2.0 min, respectively (MKJ-J1-3, Qingdao Makewave Microwave Applied Technology Co., Ltd., Shandong, China). The moisture content was detected by a moisture meter (XY-105 W, Qingdao Tuo Ke Instruments Factory Co., Ltd., China), and all dried leaves reached approximately 5% (wet basis). The loquat leaves prepared by different drying techniques were grounded into powder, and then passed through a 60-mesh sieve before use.

2.3. Preparation of polysaccharides from loquat leaves (LLPs)

The hot-water extraction of LLPs was carried out according to our reported method [9]. Polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800 were obtained, and named as LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, respectively.

2.4. Structural characterization of LLPs

2.4.1. Preliminary properties of LLPs

The proteins, uronic acids, total polysaccharides, and total phenolics in LLPs were analyzed by the Bradford's method, the *m*-hydroxydiphenyl method, the phenol-sulfuric acid method, and the Folin-Ciocalteu assay according to our previous studies [18,19], respectively.

2.4.2. Determination of molecular weights and constituent monosaccharides of LLPs

The absolute molecular weights (M_w) and polydispersities (M_w/M_n) of LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8 were detected by high performance size exclusion chromatography, which equipped with the multi angle laser light scattering and refractive index detector (HPSEC-MALLS-RID, Wyatt Technology Co., Santa Barbara, CA, USA) [9]. TSKgel G6000PWXL (300 mm × 7.8 mm, i.d.) and TSKgel G4000PWXL (300 mm × 7.8 mm, i.d.) were used in series at 30 °C.

The analysis of constituent monosaccharides of LLPs was carried out by the high performance liquid chromatography (HPLC, Agilent Technologies, Santa Clara, CA, USA) on the basis of our previously reported method [9].

2.4.3. Fourier transform-infrared spectroscopy analysis of LLPs

Polysaccharides extracted from loquat leaves prepared by different drying techniques were ground with KBr powder, pressed into a 1-mm pellet and measured by a Nicolet iS 10 FT-IR (ThermoFisher scientific, Waltham, MA, USA) according to our previously reported method [9]. The determination of degree of esterification (DE) was based on the band areas at 1700–1750 cm^{-1} (esterified uronic acids) and 1600–1630 cm^{-1} (free uronic acids). Afterwards, the DE was calculated by the equation as follows,

$$\mathrm{DE}\ (\%) = \left(\frac{A_{1740}}{A_{1740} + A_{1620}}\right) \times 100$$

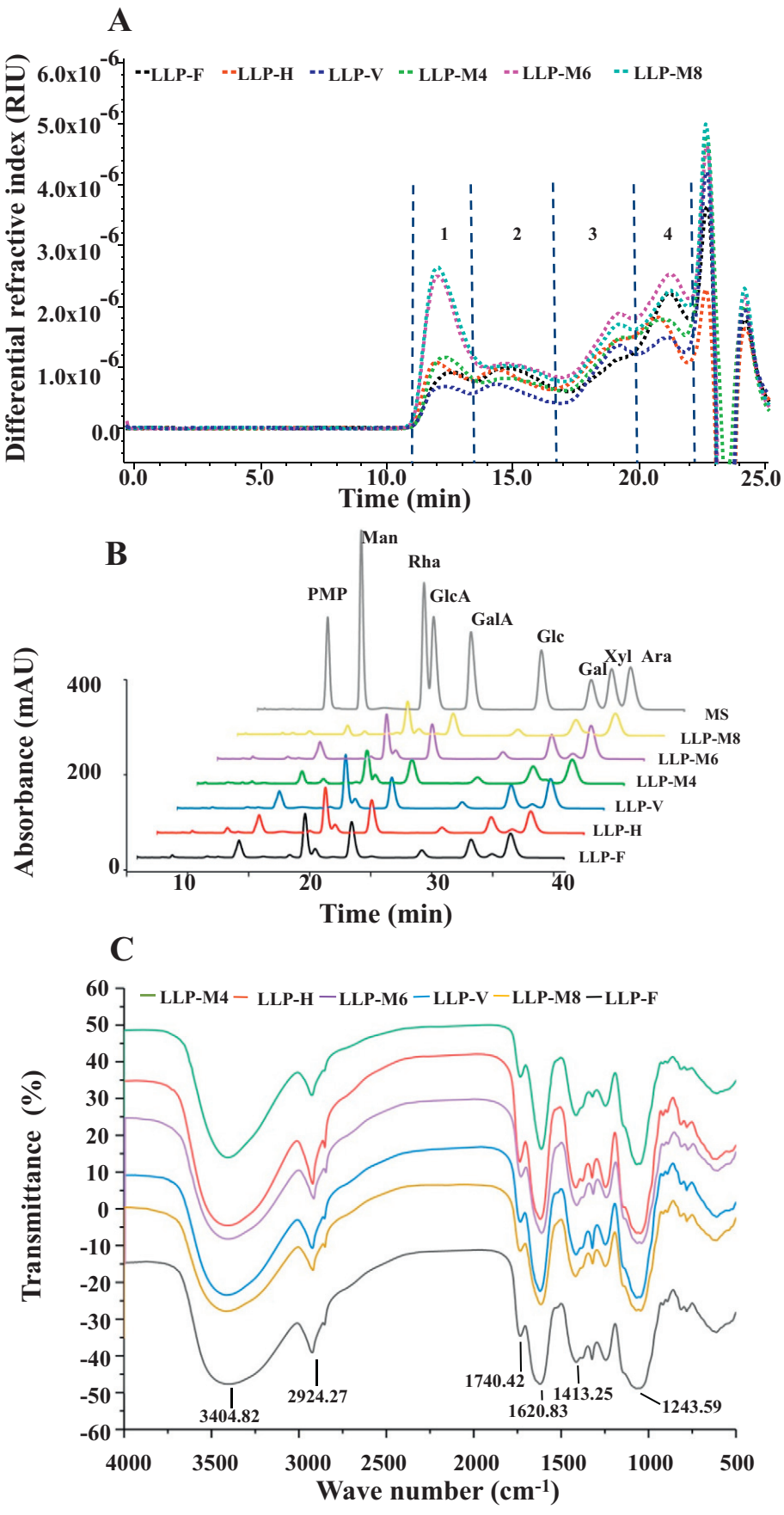
2.5. Rheological measurements of LLPs

Rheological properties of LLPs were measured by our reported method [20]. The rheological properties of LLPs at the concentration of

Table 1
Chemical compositions of LLPS.

	LLP-F	LLP-H	LLP-V	LLP-M4	LLP-M6	LLP-M8
Extraction yields (%)	3.41 ± 0.91^{c}	2.87 ± 0.99^{d}	3.62 ± 0.14^{b}	3.67 ± 1.41^{b}	3.56 ± 0.71^{b}	4.26 ± 0.21^{a}
Total polysaccharides (%)	78.10 ± 0.76^{cd}	76.14 ± 2.95^{cd}	81.82 ± 1.67^{ab}	75.69 ± 1.49^{d}	79.53 ± 2.05^{bc}	84.03 ± 3.39^{a}
Total uronic acids (%)	39.09 ± 0.54^{a}	34.14 ± 0.79^{b}	28.82 ± 0.81^{d}	33.19 ± 0.52^{bc}	30.73 ± 1.68^{c}	33.39 ± 0.31^{bc}
Total phenolics (mg GAE/g)	12.47 ± 0.58^{c}	6.46 ± 0.71^{d}	11.95 ± 0.86^{c}	18.10 ± 0.44^{a}	15.34 ± 0.36^{b}	16.35 ± 0.15^{b}
Total proteins (%)	3.89 ± 0.49^{a}	3.05 ± 1.24^{a}	4.39 ± 0.83^{a}	3.48 ± 1.59^{a}	4.22 ± 0.61^{a}	4.31 ± 0.54^{a}
Degree of esterification (%)	15.11 ± 0.47^{c}	21.87 ± 0.45^{a}	11.11 ± 0.72^{d}	14.61 ± 0.24^{c}	18.56 ± 0.39^{b}	14.58 ± 0.41^{c}

LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively; values represent mean ± standard deviation, and superscripts a-d differ significantly ($p < 0.05$) among LLPs.


A
LLP-F
LLP-H
LLP-V
LLP-M4
LLP-M6
LLP-M8
Differential refractive index (RIU)
6.0x10-6
5.0x10-6
4.0x10-6
3.0x10-6
2.0x10-6
1.0x10-6
0.0
1
2
3
4
0.0
5.0
10.0
15.0
20.0
25.0
Time (min)
B
Man
Rha
PMP
GlcA
GalA
Glc
Gal
Xyl
Ara
MS
LLP-M8
LLP-M6
LLP-M4
LLP-V
LLP-H
LLP-F
Absorbance (mAU)
400
200
0
10
20
30
40
Time (min)
C
LLP-M4
LLP-H
LLP-M6
LLP-V
LLP-M8
LLP-F
Transmittance (%)
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
3404.82
2924.27
1740.42
1620.83
1413.25
1243.59
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)

0.4% (w/v) were determined at 25 °C by a Discovery Hybrid Rheometer-1 (DHR-1, TA instruments, New Castle DE, USA) coupled with a parallel steel plate (40 mm diameter, 1.0 mm gap).

2.6. In vitro binding properties of LLPs

The assay of *in vitro* binding capacities of LLPs was carried out according to our previously reported methods [9]. 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.

2.7. In vitro antioxidant activities of LLPs

ABTS, DPPH, nitric oxide (NO) scavenging activities, and ferric reducing antioxidant powers of LLPs were measured by our previously reported methods with minor modifications [19,21]. The LLPs were detected at five different concentrations (2.0 to 6.0 mg/mL) against distilled water as blank control, and the BHT or vitamin C was used as the positive control. Afterwards, a log-regression curve was set up for calculating IC_{50} values (mg/mL).

2.8. In vitro α-amylase and α-glucosidase inhibitory effects of LLPs

α-Amylase and α-glucosidase inhibitory effects of LLPs were also carried out based on our previously reported methods [9]. The positive control was acarbose. The LLPs were measured at five different concentrations (0.1 to 0.5 mg/mL), and then a log-regression curve was set up for calculating IC_{50} values (μg/mL).

2.9. Statistical analysis

All the data were expressed as the means ± standard deviations, while the experiments were performed in triplicate. Statistical analysis was taken by Origin 9.0 software (OriginLab Corporation, Northampton, MA, USA). The statistical significances were carried out by one-way analysis of variance (ANOVA), and were considered when $p < 0.05$ evaluated by the Duncan's multiple range test.

3. Results and discussions

3.1. Physicochemical characteristics of LLPs

3.1.1. Chemical compositions of LLPs

The contents of moisture which existed in loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800 ranged from (2.39 ± 0.45)% to (4.75 ± 0.34)%. As shown in Table 1, the extraction yields of LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8 ranged from (2.87 ± 0.99)% to (4.26 ± 0.21)%, which were similar with the previous study (ranged from 3.62% to 5.29%) [9]. The most outstanding extraction yield was determined in LLP-M8 within all LLPs, which might be related to the low moisture content of loquat leaves prepared by MD at the microwave power of 800 W. Furthermore, it has been confirmed that the increasing of the drying temperature and drying time may observed a considerable decrease of the polysaccharide yields [22]. Results suggested that the property of low moisture contents of loquat leaves dried by microwave drying with a short drying time is a good fit for large scale applications in food industry [23]. The contents of total polysaccharides in LLPs ranged from (75.69 ± 1.49)% to (84.03 ± 3.39)%, which were quiet close to the previous study (ranged from 73.50% to 83.07%) [9]. A few proteins were detected in LLPs, which ranged from (3.05 ± 1.24)% to (4.39 ± 0.83)%. Results showed that polysaccharides were the major bioactive components in LLPs. Furthermore, the contents of uronic acids in LLPs ranged from (28.82 ± 0.81)% to (39.09 ± 0.54)%, which were almost the same as the previous study (ranged from 27.04% to 41.46%) [9]. LLP-F had the highest content of uronic acids among all LLPs, which probably attributed to its lowest oxygen concentration during FD drying [24]. Furthermore, it has been confirmed that the relatively high contents of uronic acids in LLPs indicating the presence of pectin-like acidic polysaccharides in the leaves [9,18]. A few phenolics were still detected in LLPs while most of them were taken away by ethanol extraction and ethanol precipitation. The contents of total phenolics in LLPs ranged from 6.46 ± 0.70 to 18.10 ± 0.44 mg GAE/g. Results indicated that natural polyphenolic-polysaccharide conjugates might exist in LLPs [20]. The content of total phenolics in LLP-M4 was the highest among all LLPs, while the lowest content of total phenolics was measured in LLP-H. It might due to the fact that both the long drying time and high drying temperature could lead to the degradation of phenolics during HD drying [15].

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

	LLP-F	LLP-H	LLP-V	LLP-M4	LLP-M6	LLP-M8
$M_w \times 10^6$ (Da)						
Fraction 1	5.283 ± 0.056^d	7.063 ± 0.175^b	5.555 ± 0.150^c	5.631 ± 0.093^c	6.944 ± 0.128^b	7.712 ± 0.203^a
Fraction 2	0.802 ± 0.020^f	2.205 ± 0.103^c	1.127 ± 0.046^e	1.342 ± 0.032^d	3.247 ± 0.177^a	2.423 ± 0.152^b
Fraction 3	0.214 ± 0.014^d	0.451 ± 0.032^b	0.243 ± 0.016^d	0.343 ± 0.012^c	0.599 ± 0.042^a	0.504 ± 0.050^b
Fraction 4	0.046 ± 0.002^f	0.169 ± 0.002^b	0.051 ± 0.002^e	0.086 ± 0.001^d	0.178 ± 0.002^a	0.115 ± 0.004^c
M_w/M_n						
Fraction 1	1.27	1.15	1.21	1.10	1.05	1.07
Fraction 2	1.14	1.10	1.06	1.10	1.29	1.12
Fraction 3	1.21	1.19	1.47	1.31	1.31	1.32
Fraction 4	1.34	1.18	1.10	1.36	1.33	1.05
Monosaccharides and molar ratios						
Rhamnose	1.00	1.00	1.00	1.00	1.00	1.00
Galacturonic acid	0.95	0.85	0.69	1.00	0.85	0.92
Arabinose	0.86	0.75	0.88	1.17	1.09	1.10
Galactose	0.75	0.64	0.81	1.76	0.95	1.66
Glucuronic acid	0.14	0.12	0.10	0.16	0.11	0.15
Glucose	0.29	0.21	0.20	0.36	0.25	0.27
Xylose	0.11	0.10	0.10	0.12	0.14	0.10
Mannose	0.35	0.36	0.31	0.07	0.33	0.06

LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively; values represent mean ± standard deviation; superscripts a-f differ significantly ($p < 0.05$) among LLPs.

3.1.2. Molecular weights of LLPs

The molecular weights of natural polysaccharides are generally related to their biological activities [25]. Hence, the evaluation of molecular weights was essential, which could investigate the effects of different drying techniques on the molecular weights of LLPs. Fig. 1A demonstrated that the HPSEC-RID chromatograms of LLPs extracted from loquat leaves prepared by different drying methods were quiet similar. Four fractions were detected in LLPs, and the molecular weights of fraction 1, fraction 2, fraction 3, and fraction 4 in LLPs ranged from 5.283×10^6 to 7.712×10^6 Da, from 0.802×10^6 to 3.247×10^6 Da, from 0.214×10^6 to 0.599×10^6 Da, and from 0.046×10^6 to 0.178×10^6 Da, respectively (Table 2), which were

Fig. 1. High performance size exclusion chromatograms (A), high performance liquid chromatography profiles (B), and FT-IR spectra (C) of LLPs. LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively; PMP, 1-phenyl-3-methyl-5-pyrazolone; Rha, rhamnose; GalA, galacturonic acid; Ara, arabinose; Gal, galactose; GlcA, glucuronic acid; Glc, glucose; Xyl, xylose.

close to the previous reported study [9]. Molecular weights of LLPs were varied by different drying techniques. Results suggested that the molecular weights of LLP-F were significantly lower than others, while the molecular weights of polysaccharide fraction 1 in LLP-M8 and LLP-H reached up to 7.712×10^6 Da and 7.063×10^6 Da, respectively. The similar results have been found in polysaccharides extracted from the bamboo shoot, which revealed that the polysaccharides easily aggregated under the drying condition of a relatively high temperature [26]. Additionally, the polydispersities of polysaccharide fractions 1 to 4 in LLPs matched with the HPSEC chromatograms, which ranged from 1.05 to 1.27, from 1.06 to 1.29, from 1.19 to 1.47, and from 1.05 to 1.36, respectively.

3.1.3. Constituent monosaccharides of LLPs

Monosaccharide is the natural basic unit that can determine the structures and characteristics of polysaccharide. In order to well understand chemical structures of LLPs, analysis of constituent monosaccharides was carried out. Fig. 1B demonstrated that the HPLC-UV profiles of LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8 were similar, and Rha, GalA, Ara, Gal, GlcA, Glc, Xyl, and Man were measured in LLPs. Results indicated that constituent monosaccharides in LLPs were not affected by different drying techniques. Table 2 summarized the molar ratios of Rha, GalA, Ara, Gal, GlcA, Glc, Xyl and Man in LLPs, which were varied by different drying techniques. Furthermore, the dominant monosaccharides in LLPs were Rha, GalA, Ara, and Gal, which were

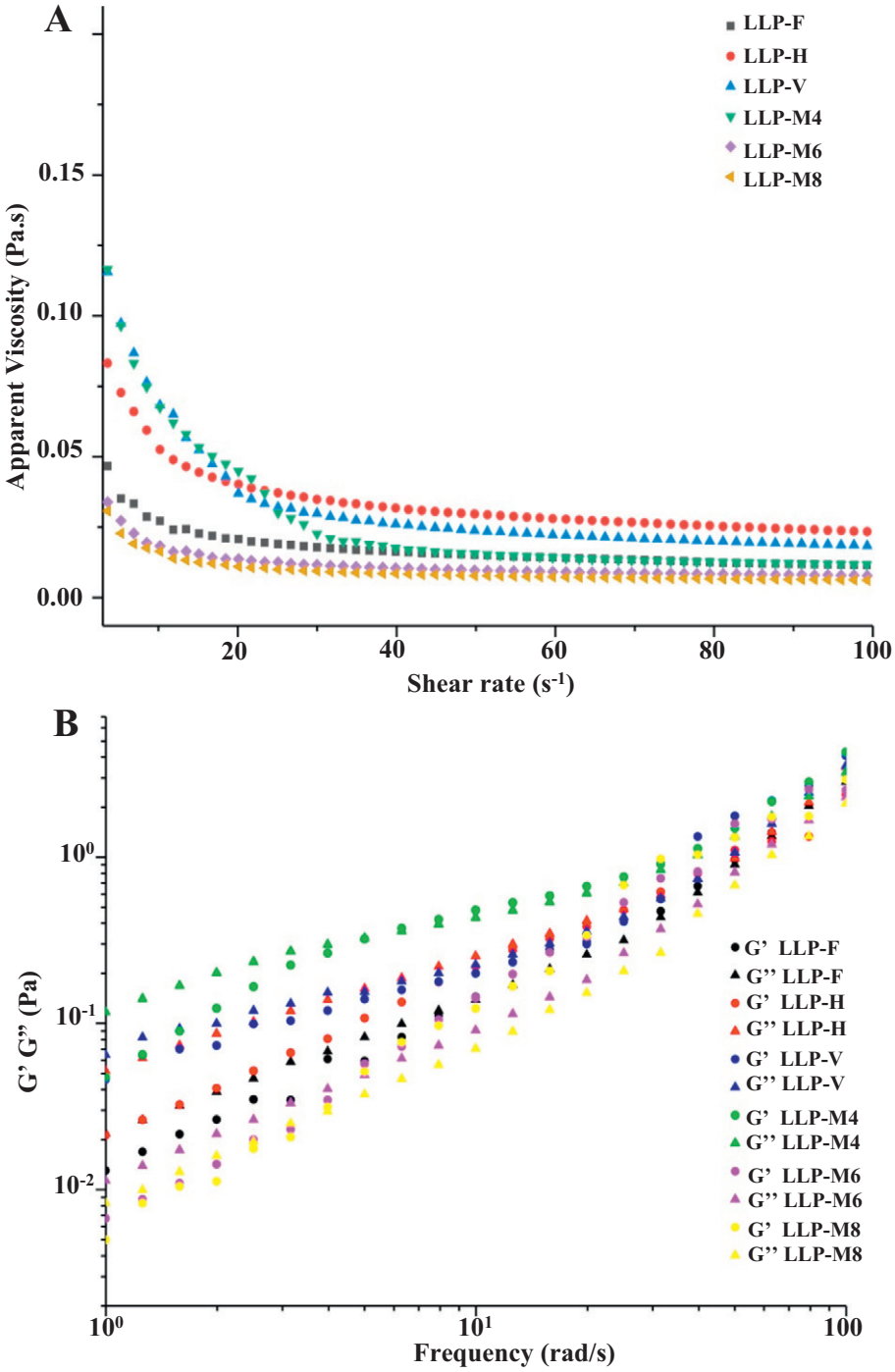


Fig. 2. Dependence of apparent viscosity on the shear rate (A) and plots of storage modulus G′ and loss modulus G″ against frequency (B) for LLPs. LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively.

similar with the previously reported study [9]. Results suggested that pectic-polysaccharides existed in loquat leaves, and rhamnogalacturonan I (RG I), homogalacturonan (HG), and arabinogalactan (AG II) might exist in LLPs according the monosaccharide compositions [9].

3.1.4. FT-IR spectra and esterification of LLPs

The structural features of LLPs were also determined by the FT-IR spectroscopy analysis. As presented in Fig. 1C, the FT-IR spectra of LLPs from loquat leaves prepared by different drying techniques were similar, which indicated that they exhibited similar structures. The strong absorption peaks at 3404.82 cm^{-1} and 2924.27 cm^{-1} were the characteristic stretching absorption peaks of O—H and C—H absorption [27]. The absorption peak at 1740.42 cm^{-1} was the stretching vibration of the esterified carboxylic groups [9]. Moreover, the strong absorption peak at 1620.83 cm^{-1} was the C═O, demonstrating the LLPs were acidic polysaccharides [18,25]. The signal at 1413.25 cm^{-1} was related to C—H or O—H [21]. Furthermore, the signal at 1243.59 cm^{-1} was the C-O-C, consisting with the existence of -OCH_3 [9]. The absorption peaks in 800–1200 cm^{-1} named fingerprint region were quiet similar, suggesting that the structures of LLPs extracted from loquat leaves prepared by different drying techniques were similar [28]. 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 (Table 1). The degree of esterification (DE) of LLPs was also conducted by FT-IR spectroscopy analysis. LLP-H exhibited the highest DE (21.87%), followed by lower DE in LLP-M6 (18.56%), LLP-F (15.11%), LLP-M4 (14.61%), and LLP-M8 (14.58%), and the lowest DE was determined in LLP-V (11.11%). Previous studies have demonstrated that the higher antioxidant activity of natural polysaccharides generally possess the lower DE [27,29].

3.2. Rheological properties of LLPs

The ideal functional properties of natural polysaccharides, such as foaming, gelling properties, thickening, and emulsifying, have been detected and discussed for a long time [30,31]. Therefore, the effect of different drying methods on the apparent viscosities of LLPs was investigated. As shown in Fig. 2, the apparent viscosities of LLPs were affected by the shear rate. The non-Newtonian shear thinning behaviors could be found in LLPs solutions at low shear rate range (0.01–50 s^{-1}), while closely Newtonian flow behavior were found at high shear rate rang (50–100 s^{-1}). It has been confirmed that the shear thinning behavior of polysaccharides could associated with the disentanglement of molecular chains in solution [31,32]. The outstanding apparent viscosities were determined in LLP-H, LLP-V, and LLP-M4, while the lowest apparent viscosity was measured in LLP-M8. This could be related to their high molecular weights, wide polydispersities, and high DEs [33]. Results demonstrated that different drying techniques could affect the apparent viscosities of LLPs, and the LLPs could be utilized as a thickener in the food industry.

Polysaccharides are viscoelastic materials, the measurement of solid and liquid characters of polysaccharides could be taken by dynamic measurements [34]. The constant strain of 10% has been chosen for the frequency sweep, which belonged to linear viscoelastic region. As shown in Fig. 2, 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 G″ of LLPs was higher than the G′ at low frequency, indicating liquid-like behavior [20]. The G′ began to exceed the G″ beyond a certain frequency, expressing a significant trend for weak gel like behavior [35]. Furthermore, compared with LLP-F, the angular frequencies at crossover point of G″ and G′ of LLP-H, LLP-V, and LLP-M4 were increased, while the angular frequencies at crossover point of G″ and G′ of LLP-M6 and LLP-M8 were decreased. This phenomenon was accordance with the apparent viscosities of LLPs. Results demonstrated that different drying techniques also influenced the G″ and G′ of LLPs. All results signified that pectic polysaccharides from loquat leaves prepared by different drying methods could be used as thickening agents and gelling agents in the food industry.

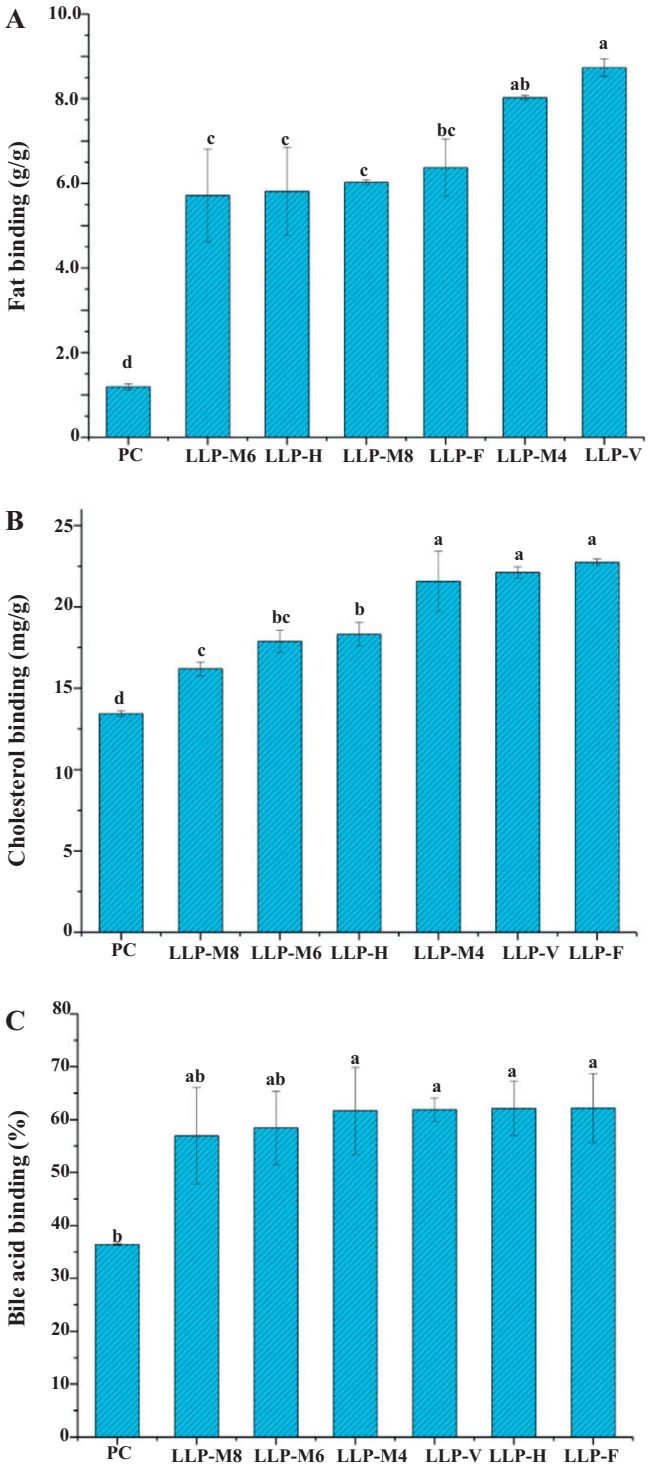


Fig. 3. The fat binding (A), cholesterol binding (B), and bile acid binding capacities (C) of LLPs. PC, positive control group; LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively; cellulose was used as a positive control in fat binding and cholesterol binding capacity assay, respectively, and cholestyramine was used as a positive control in bile acid binding capacity assay; values represent mean ± standard deviation, and superscripts a-d differ significantly ($p < 0.05$) among LLPs; statistical significances were carried out by ANOVA, followed by Duncan's test.

3.3. In vitro binding properties of LLPs

Some obesity issues which were related to cancer and diabetes could be caused by over absorption of fat, cholesterol, and bile acid [4]. Hypolipidemic and hypocholesterolemic effects of natural polysaccharides are generally related to their binding capacities such as fat binding, cholesterol binding, and bile acid binding [36]. Previous studies have shown that LLPs exhibited strong *in vitro* binding capacities [9]. Hence, the comparison of the effects of different drying techniques on the *in vitro* fat, cholesterol, and bile acid binding capacities of LLPs was essential. As shown in Fig. 3, the fat binding, cholesterol binding, and bile acid binding capacities of LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8 ranged from 5.71 ± 1.10 to 8.73 ± 0.21 g/g, from 16.19 ± 0.42 to 22.74 ± 0.14 mg/g, and from (56.93 ± 9.16)% to (62.18 ± 6.52)%, respectively. The higher binding properties can be easily found in LLPs when compared with the positive controls. Additionally, the LLP-M4 and LLP-V exerted outstanding binding capacities among all tested samples, which might be related to their high viscosities, wide molecular weight distributions, and high molecular weights [9,18,20,27,36]. Results showed that different drying techniques significantly influence the *in vitro* binding capacities of polysaccharides from loquat leaves. Indeed, the MD-400 method had the advantages of high drying rate, easy process control, and homogeneous energy delivery on the material, which could be an efficient drying technique before extraction of LLPs from loquat leaves with remarkable binding capacities.

3.4. In vitro antioxidant activities of LLPs

Previous studies have demonstrated that loquat leaves exert strong antioxidant activities [7]. Therefore, the effects of different drying techniques on the antioxidant activities of LLPs were investigated and compared in this study. Fig. 4A, B, C, and D showed the ABTS, DPPH, and

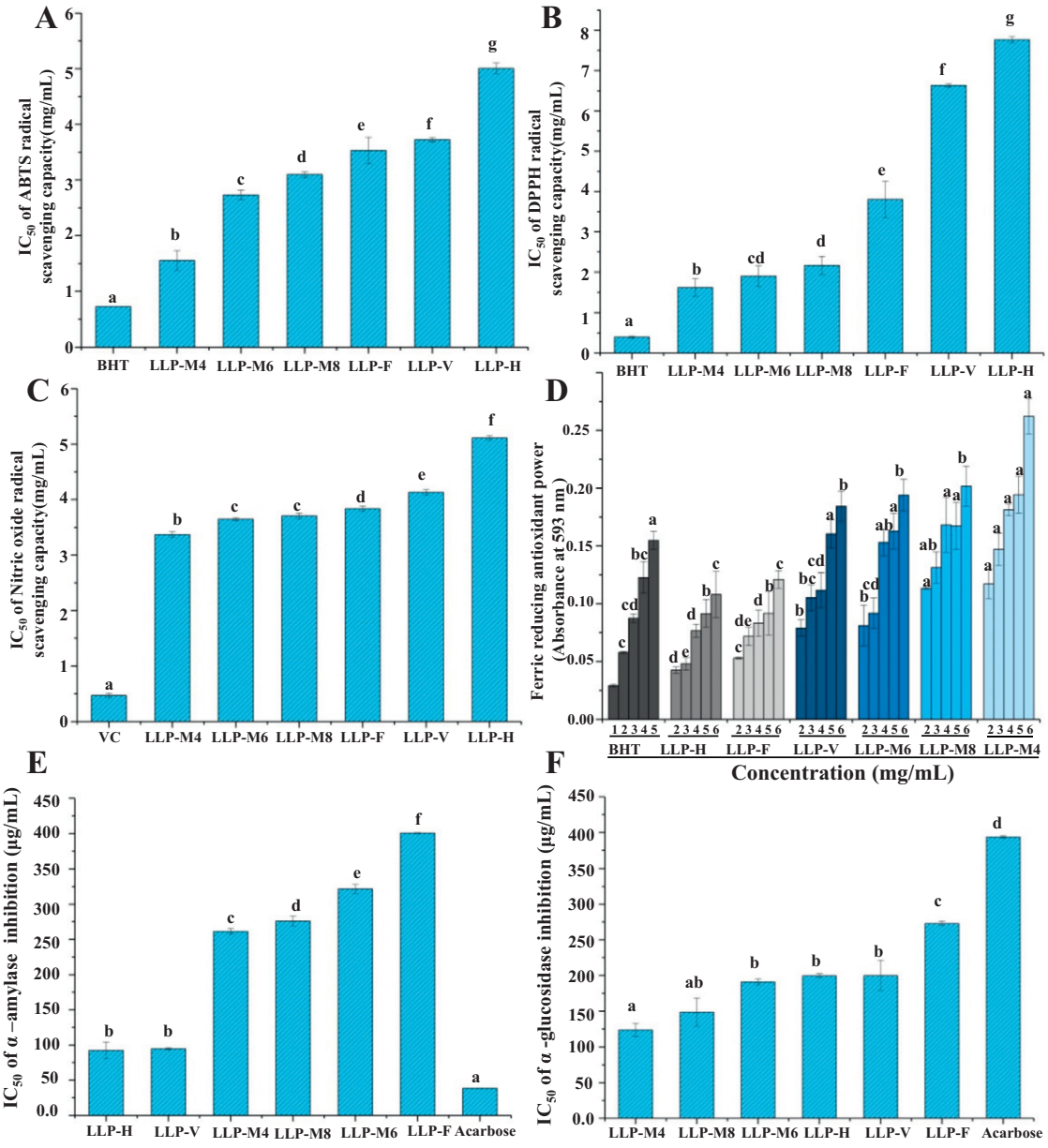


Fig. 4. ABTS (A), DPPH (B), and nitric oxide (C) radical scavenging activities, and ferric reducing antioxidant power (D), as well as inhibitory effects on α-amylase (E) and α-glucosidase (F) of LLPs. LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8, polysaccharides extracted from loquat leaves prepared by FD, HD, VD, MD-400, MD-600, and MD-800, respectively; the error bars are standard deviations; significant ($p < 0.05$) differences are shown by data bearing different letters (a-g); statistical significances were carried out by ANOVA and Duncan's test.

nitric oxide (NO) radical scavenging activities, and ferric reducing antioxidant powers (FRAP) of LLPs. Compared with the positive controls, LLPs exhibited moderate ABTS, DPPH, and NO radical scavenging activities (Figs. 4A, 3B, and C). The IC_{50} values of ABTS, DPPH, and NO radical scavenging activities of LLPs ranged from 1.55 ± 0.18 mg/mL to 5.01 ± 0.10 mg/mL, from 1.62 ± 0.22 mg/mL to 7.77 ± 0.08 mg/mL, and from 3.37 ± 0.05 mg/mL to 5.11 ± 0.04 mg/mL, respectively. Furthermore, LLPs also showed strong FRAP when compared with the positive control (Fig. 4D). Results showed that different drying techniques significantly affected the antioxidant activities of LLPs, and the same phenomenon can be also found in the *Hovenia dulcis* and okra [37,38]. The highest antioxidant activities were observed in LLP-M4 among all LLPs, while LLP-H exhibited the lowest antioxidant activities. Results indicated that the MD-400 method could be a potentially efficient drying method before extracting polysaccharides from loquat leaves with relatively high antioxidant activity. Moreover, it is recognized that the antioxidant activities of natural polysaccharides may be associated with their content of total phenolic compounds, content of unmethylated galacturonic acids, and low molecular weights [19,39,40]. Therefore, the higher antioxidant activities of LLP-M4 obtained by microwave drying at the power of 400 W might be related to its relatively lower molecular weights, higher content of unmethylated galacturonic acids, and higher content of total phenolic compounds (Tables 1 and 2).

3.5. *In vitro* α-amylase and α-glucosidase inhibitory activities of LLPs

One of the major strategies to counteract metabolic alterations associating with type 2 diabetes is the inhibition of α-glucosidase and α-amylase activities [28]. It has been confirmed that some pectic-polysaccharides possess superlative *in vitro* anti-diabetic effects [3,11,18]. Previous studies have also demonstrated that pectic-polysaccharides extracted from loquat leaves exhibit outstanding *in vitro* α-amylase and α-glucosidase inhibitory effects [9]. Therefore, comparison of the effects of different drying techniques on the *in vitro* α-amylase and α-glucosidase inhibitory effects of LLPs were necessary. As shown in Fig. 3E and F, excellent inhibitory effects on α-amylase and α-glucosidase of LLPs have been detected. The IC_{50} values of α-amylase inhibition of LLP-F, LLP-H, LLP-V, LLP-M4, LLP-M6, and LLP-M8 ranged from 92.33 ± 11.59 μg/mL to 400.67 ± 0.57 μg/mL, which recommended that the α-amylase inhibitory effects of LLPs varied by different drying methods. The strongest α-amylase inhibitory effects were determined in LLP-H and LLP-V, while the inhibitory effect of LLP-F was the weakest. In addition, Compared with the positive control (Acarbose, IC_{50} = 36.85 ± 0.28 μg/mL), LLP-H and LLP-V also exhibited remarkable inhibitory effects on α-amylase. Moreover, the IC_{50} values of α-glucosidase inhibitory effects of polysaccharides extracted from the loquat leaves prepared by different methods ranged from 123.67 ± 9.29 μg/mL to 273.00 ± 2.83 μg/mL, which were stronger than that of the positive control (Acarbose, IC_{50} = 393.76 ± 3.57 μg/mL). The strongest α-glucosidase inhibitory effects were determined in LLP-M4 and LLP-M8 among all LLPs, while the weakest α-glucosidase inhibitory effect was also detected in LLP-F. The highest α-amylase inhibitory effects were determined in LLP-H and LLP-V among all LLPs, while the LLP-M4 and LLP-M8 exerted the strongest α-glucosidase inhibitory effects. Different mechanisms of action between α-glucosidase and α-amylase might lead to this condition [11]. It has been confirmed that the reaction between substrate and enzymes probably affected by surroundings, and a non-competitive interaction was usually considered for the inhibitory effects of pectic-polysaccharides on digestive enzymes [41]. Furthermore, the outstanding *in vitro* α-amylase and α-glucosidase inhibitory effects of LLPs might be associated with their high contents of uronic acids, high degrees of esterification, and high molecular weights [9,11,41]. Meanwhile, LLP-M4 also had the relatively high content of total phenolics, which might also contribute to its inhibitory effects on α-amylase and α-glucosidase [20].

4. Conclusions

In this study, results suggested that different drying techniques had noticeable influences on the physicochemical characteristics and bioactivities of LLPs. Results showed that the microwave drying could be an efficient drying technique before extraction of LLPs with relatively high bioactivities, which could broaden their potential applications in the functional food and pharmaceutical industries.

CRediT authorship contribution statement

Yuan Fu: Data curation, Formal analysis, Investigation, Resources, Software, Writing - original draft. **Kang-Lin Feng:** Formal analysis, Investigation, Validation, Resources, Software, Writing - original draft. **Si-Yu Wei:** Formal analysis, Investigation. **Xian-Rong Xiang:** Formal analysis, Investigation. **Ye Ding:** Formal analysis, Investigation. **Hua-Yu Li:** 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.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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