

## Article

# Structural and Biological Properties of Water Soluble Polysaccharides from Lotus Leaves: Effects of Drying Techniques

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**Abstract:** In the present study, the influence of five drying techniques on the structural and biological properties of polysaccharides from lotus leaves (LLPs) was investigated. Results revealed that the yields, contents of basic chemical components, molecular weights, and molar ratios of compositional monosaccharides of LLPs varied by different drying technologies. Low molecular weight distributions were observed in polysaccharides obtained from lotus leaves by hot air drying (LLP-H), microwave drying (LLP-M), and radio frequency drying (LLP-RF), respectively. The high contents of bound polyphenolics were measured in LLP-H and LLP-M, as well as polysaccharides obtained from lotus leaves by vacuum drying (LLP-V). Furthermore, both Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra of LLPs were similar, indicating that drying technologies did not change their basic chemical structures. Besides, all LLPs exhibited obvious biological properties, including in vitro antioxidant capacities, antiglycation activities, and inhibitory effects on  $\alpha$ -glucosidase. Indeed, LLP-H exhibited higher 2,2-azidobisphenol (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging ability ( $IC_{50}$  values, LLP-H,  $0.176 \pm 0.004$  mg/mL; vitamin C,  $0.043 \pm 0.002$  mg/mL) and 2,2-diphenyl-1-(2,4,6-trinitrate phenyl) hydrazine radical scavenging ability ( $IC_{50}$  values, LLP-H,  $0.241 \pm 0.007$  mg/mL; butylated hydroxytoluene,  $0.366 \pm 0.010$  mg/mL) than others, and LLP-M exerted stronger antiglycation ( $IC_{50}$  values, LLP-M,  $1.023 \pm 0.053$  mg/mL; aminoguanidine,  $1.744 \pm 0.080$  mg/mL) and inhibitory effects on  $\alpha$ -glucosidase ( $IC_{50}$  values, LLP-M,  $1.90 \pm 0.02$   $\mu$ g/mL; acarbose,  $724.98 \pm 16.93$   $\mu$ g/mL) than others. These findings indicate that both hot air drying and microwave drying can be potential drying techniques for the pre-processing of lotus leaves for industrial applications.

**Keywords:** lotus leaf; polysaccharides; drying techniques; structural properties; antioxidant; antiglycation;  $\alpha$ -glucosidase inhibition



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## 1. Introduction

*Nelumbo nucifera* Gaertn. (lotus), an aquatically perennial plant, belongs to the family Nelumbonaceae, which is an edible and medicinal plant widely consumed in China [1]. Generally, lotus leaf is consumed as a popular tea material, which also has a long history, being utilized as a traditional Chinese herb for treating diverse diseases, such as haematemesis, epistaxis, hyperlipidaemia, and obesity [2]. Recently, it has also attracted increasing attention as a potential functional food due to its diverse health-promoting effects, such as antioxidant, anti-hyperglycemic, anti-proliferative, anti-inflammatory, anti-obesity,

and hepatoprotective activities [1–3]. Usually, these health-promoting effects are correlated with different bioactive compounds that exist in lotus leaves, such as polysaccharides, flavonoids, alkaloids, essential oils, and triterpenoids [1]. Especially, polysaccharide is one of the most abundant components in the water decoction of lotus leaves, which possesses several health benefits, including obvious anti-osteoporotic, immunostimulatory, antioxidant, and antidiabetic activities [3–7]. Thus, polysaccharides from lotus leaves (LLPs) possess potential applications in the pharmaceutical and functional food industries.

Freshly picked lotus leaves usually contain high moisture and are thus easily spoiled, leading to the loss of beneficial ingredients. Drying can be used to quickly and effectively protect the beneficial ingredients and extend the shelf-life of medicinal and edible plants [8]. This is considered the most common and basic strategy for the pre-processing of lotus leaves. Usually, the dried lotus leaves are used as a popular tea and a traditional Chinese herb with multiple health-promoting effects in China [9]. Thus, the drying method plays a key role in the production of lotus leaf product. Generally, freeze drying (FD), vacuum drying (VD), microwave drying (MD), and hot air drying (HD), as well as radio frequency drying (RFD) are commonly used in food processing. These techniques have their own features in terms of efficiency, convenience, time, and cost. FD based on the principle of sublimation can retain the original nutrients, active ingredients, and color of postharvest plants. HD is cheap and easy to control because the postharvest plants are dried by continuous flowing hot air [10]. The plants after VD treatment can effectively reduce the structural changes because of the relatively low temperature and absence of oxygen [11]. MD has the advantages of fast drying, uniform sample energy transfer, and easy control of the drying process [12]. RFD has the advantages of uniform drying rate, short drying time, and good product quality [13]. Several studies have found that drying technologies have an important effect on the chemical properties and biological activities of plant polysaccharides [14–16]. A previous study revealed that oven drying (55–60 °C) and microwave drying (680–850 W) can affect the quality and antioxidant activity of lotus leaves [17].

However, whether the drying technologies can affect the chemical properties, such as molecular weight, compositional monosaccharides, and chemical components of polysaccharides from lotus leaves remains unknown. Indeed, the effect of drying technologies on the antioxidant and antidiabetic activities of polysaccharides from lotus leaves is also unknown. Therefore, different drying techniques were applied for the pre-processing of lotus leaves in this study, and the effects of drying techniques on the physicochemical (basic chemical components, molecular weight distribution, monosaccharide composition, and chemical structure) and biological properties (antioxidant capacities, antiglycation activities, and inhibitory effects on  $\alpha$ -glucosidase) of LLPs were carefully evaluated.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

Lotus leaves (*Nelumbo nucifera* cv. Elian 6) were collected from a lotus planting base (Renshou Rennong Lotus Professional Cooperative, Renshou, Sichuan, China) on 28 May 2019 before the blooming of lotus. Then, lotus leaves were washed and cut into about 4 square centimeters before drying.

Monosaccharide standards, aminoguanidine, 1-phenyl-3-methyl-5-pyrazolinone (PMP), trifluoroacetic acid (TFA), 2,2-azidobisphenol (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), m-hydroxybiphenyl, 2,2-diphenyl-1-(2,4,6-trinitrate phenyl) hydrazine (DPPH), Griess reagent, acarbose,  $\alpha$ -glucosidase from *Bacillus stearothermophilus*, and 4-nitrophenyl  $\beta$ -D-pyran glucoside were obtained from Sigma-Aldrich. All other chemicals and solvents were of analytical grade.

### 2.2. Drying Processes

The RFD, HD, FD, MD, and VD techniques were applied for the pre-processing of lotus leaves. The RFD was performed by using a pilot-scale 8 kW, 27.12 MHz RF heating system (Shijiazhuang Huas Jiyuan High Frequency Equipment Co., Ltd., Shijiazhuang,

China) at the power of 700 W and temperature of 60 °C for 1.5 h according to the procedure described by a previous study [18]. Other drying methods were also carried out according to a previous study [15]. Briefly, the HD was carried out with hot air at 75 °C for 3.5 h (101A-3, Shanghai Experimental Instrument Factory Co., Ltd., Shanghai, China). For the FD, lotus leaves were frozen at −80 °C and then dried using a freeze dryer for 48 h (SCIENTZ-12N, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). The VD was conducted at 50 °C for 21 h (DZF-6050, Shanghai San Fa Scientific Instruments Factory Co., Ltd., Shanghai, China). The MD was performed at 85 °C and 400 W for 13 min (MKJ-J1-3, Qingdao Makewave Microwave Applied Technology Co., Ltd., Shandong, China). The drying experiments lasted until the moisture contents of lotus leaves were less than approximately 10% (wet basis) as detected by a moisture meter. After drying, the lotus leaves were stored at −20 °C before hot water extraction.

### 2.3. Preparation of LLPs

Polysaccharides in lotus leaves were extracted with hot water, as previously reported [15]. Briefly, the powder of dried lotus leaves (10.0 g) was pre-treated with 80% methanol (*v/v*) twice for 1 h to remove small molecules, and then the residues were extracted twice with deionized water (300.0 mL) at 90 °C for 3 h. The extract was concentrated, precipitated with 95% ethanol (*v/v*), dialyzed (molar mass cutoff: 3.0 kDa), and lyophilized to obtain polysaccharides from lotus leaves dried by HD, MD, VD, RFD, and FD, which were then encoded as LLP-H, LLP-M, LLP-V, LLP-RF, and LLP-F, respectively.

### 2.4. Characterization of Physicochemical Properties of LLPs

#### 2.4.1. Chemical Components of LLPs

The contents of total polysaccharides, the contents of uronic acids, the contents of proteins, and the contents of bound polyphenolics in LLPs obtained by different drying technologies were detected based on the previous studies [19,20].

#### 2.4.2. Determination of Molecular Weights and Monosaccharide Compositions of LLPs

The molecular weights ( $M_w$ ) of LLPs were measured by size-exclusion chromatography followed by multi-angle laser light scattering detection and refractive index detection (SEC-MALLS-RID, Wyatt Technologies, Santa Barbara, CA, USA) as previously reported [15]. The Shodex OHpak SB-806M HQ column was applied to separate LLPs at a stable temperature of 30 °C. The monosaccharide compositions of LLPs obtained by different drying technologies were detected on a Thermo U3000 HPLC system (ThermoFisher, Waltham, MA, USA) coupled with a phenomenex gemini C18 110A column as previously reported [20].

#### 2.4.3. Analysis of FT-IR Spectra of LLPs

FT-IR spectra of LLPs were recorded on a Nicolet iS 10 FT-IR (ThermoFisher scientific, Waltham, MA, USA) in the frequency range of 4500–400  $\text{cm}^{-1}$  based on a previous study [15]. The DE value was determined according to the band areas of 1700–1750  $\text{cm}^{-1}$  and 1600–1630  $\text{cm}^{-1}$ , and calculated by the following formula,

$$\text{DE (\%)} = \left( \frac{A_{1734}}{A_{1734} + A_{1616}} \right) \times 100 \quad (1)$$

#### 2.4.4. Analysis of NMR Spectra of LLPs

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of LLPs obtained by different drying technologies were tested by using a Bruker Ascend 600 MHz spectrometer with a z-gradient probe (Bruker, Rheinstetten, Germany). The frequencies of carbon and proton were 150.90 and 600.13 MHz, respectively. The acquisition temperature, internal reference, and number of scans were 298 K, 3-(trimethylsilyl) propionic-2,2,3,3-d<sub>4</sub> acid sodium salt, and 6000, respectively.

## 2.5. Determination of In Vitro Antioxidant Activities, Antiglycation Activities, and $\alpha$ -Glucosidase Inhibitory Effects of LLPs

The in vitro antioxidant activities of LLPs obtained by different drying technologies were measured by the ABTS and DPPH radical scavenging assays, as well as the ferric reducing antioxidant power (FRAP) assay according to a previous study [16]. The  $IC_{50}$  values (mg/mL) of radical scavenging abilities were determined by a logarithmic regression curve, while the absorbance of the reaction solution at 593 nm represented the FRAP of LLPs.

The antiglycation activities of LLPs obtained by different drying technologies were determined by a BSA-glucose model, which was also conducted based on a previous study with slight modifications [14]. The samples were measured at the concentrations ranged from 0.25–4.00 mg/mL, and aminoguanidine (AG) was used as the positive control.

The  $\alpha$ -glycosidase inhibitory activities of LLPs were also tested based on a reported study [21]. LLP-H, LLP-M, LLP-V, and LLP-RF were determined at concentrations ranging from 2.0 to 6.0  $\mu$ g/mL, respectively. Besides, LLP-F was measured at concentrations ranging from 5 to 25  $\mu$ g/mL. Acarbose was used as the positive control in this study.

## 2.6. Statistical Analysis

All experiments in this study were performed in triplicate, while the data were expressed in means  $\pm$  standard deviations. Statistical significances were carried out by analysis of variance (ANOVA) plus post hoc Duncan's test using SPSS software.

## 3. Results and Discussion

### 3.1. Physicochemical Properties of LLPs Affected by Different Drying Techniques

#### 3.1.1. Basic Chemical Components of LLPs

The contents of moisture in lotus leaves after HD, MD, VD, RFD, and FD treatments were measured to be  $6.54\% \pm 0.24\%$ ,  $7.66\% \pm 0.16\%$ ,  $7.57\% \pm 0.15\%$ ,  $8.07\% \pm 0.17\%$ , and  $6.91\% \pm 0.25\%$ , respectively. The yields and basic chemical components of LLPs prepared by different drying techniques are presented in Table 1. The yields of LLPs were influenced by drying technologies, ranging from  $3.44\% \pm 0.55\%$  to  $4.57\% \pm 0.64\%$ , which were higher compared to the results (0.97–1.93%) of a previous study [6]. The yields of LLP-RF and LLP-F were higher than that of other tested LLPs. It is reported that freeze drying causes more porous microstructures and ice crystals to form cellular structures in the plant matrix to promote solvent penetration during the extraction process [22]. The yields also confirmed that the radio frequency drying technique was a valuable drying process for obtaining polysaccharides from lotus leaves. The contents of polysaccharides and proteins in LLPs were also influenced by drying technologies, which ranged from  $66.43\% \pm 1.57\%$  to  $76.35\% \pm 1.17\%$ , and from  $4.44\% \pm 0.50\%$  to  $8.76\% \pm 0.67\%$ , respectively. Results revealed that the main components in LLPs were polysaccharides. Furthermore, the contents of uronic acids in LLPs were also influenced by drying technologies, ranging from  $12.92\% \pm 0.23\%$  to  $20.29\% \pm 1.74\%$ . The highest uronic acid content was tested in LLP-F among LLPs, which might be due to the lowest oxygen concentration and the lowest temperature during freeze drying [16]. The high content of uronic acids in LLPs indicated the presence of pectic-polysaccharides in lotus leaves [14]. Moreover, some polyphenolics were still found in LLPs after methanol extraction, ethanol precipitation, and dialysis treatments. The contents of bound polyphenolics in LLPs ranged from  $47.36 \pm 2.70$  mg GAE/g to  $119.87 \pm 2.76$  mg GAE/g, which were also significantly influenced by drying technologies. The long heat treatment could reduce the porosity of samples and increase the apparent saturation and apparent affinity. Moreover, the long heat treatment could also destroy the physical structure of the sample matrix, making it easier for small molecules to combine with polysaccharides [23]. Therefore, the contents of bound polyphenolics in LLP-H, LLP-V, and LLP-M were higher than that of others. Generally, the bound polyphenolics can enhance the biological activities of natural polysaccharides [19,24].

**Table 1.** Extraction yields and chemical compositions of LLPs.

	LLP-H	LLP-M	LLP-V	LLP-RF	LLP-F
Extraction yields (%)	3.44 ± 0.55 <sup>b</sup>	3.84 ± 0.59 <sup>ab</sup>	3.70 ± 0.65 <sup>ab</sup>	4.57 ± 0.64 <sup>a</sup>	4.17 ± 0.99 <sup>ab</sup>
Total polysaccharides (%)	72.05 ± 2.27 <sup>b</sup>	68.49 ± 1.04 <sup>c</sup>	76.35 ± 1.17 <sup>a</sup>	72.01 ± 1.98 <sup>b</sup>	66.43 ± 1.57 <sup>c</sup>
Total uronic acids (%)	14.65 ± 0.66 <sup>b</sup>	12.92 ± 0.23 <sup>c</sup>	13.29 ± 1.05 <sup>bc</sup>	13.84 ± 0.40 <sup>bc</sup>	20.29 ± 1.74 <sup>a</sup>
Total polyphenolics (mg GAE/g)	119.87 ± 2.76 <sup>a</sup>	109.67 ± 1.22 <sup>b</sup>	113.37 ± 2.37 <sup>b</sup>	81.55 ± 2.87 <sup>c</sup>	47.36 ± 2.70 <sup>d</sup>
Total proteins (%)	8.76 ± 0.67 <sup>a</sup>	8.17 ± 0.36 <sup>a</sup>	8.60 ± 0.25 <sup>a</sup>	6.76 ± 0.36 <sup>b</sup>	4.44 ± 0.50 <sup>c</sup>
Degree of esterification (%)	13.34 ± 0.20 <sup>c</sup>	20.43 ± 0.57 <sup>ab</sup>	16.79 ± 0.24 <sup>bc</sup>	14.60 ± 0.57 <sup>c</sup>	22.34 ± 0.20 <sup>a</sup>

LLP-H, LLP-M, LLP-V, LLP-RF and LLP-F are polysaccharides from lotus leaves obtained by hot air drying, microwave drying, vacuum drying, radio frequency drying, and freeze drying, respectively; Values represent mean ± standard deviation, and superscripts <sup>a-d</sup> differ significantly ( $p < 0.05$ ) among LLPs; Statistical significances were carried out by ANOVA plus post hoc Duncan's test.

### 3.1.2. Molecular Weight Distributions and Monosaccharide Compositions of LLPs

According to previous studies, the biological functions of polysaccharides are usually associated with their compositional monosaccharides and molecular weights [20]. Thus, we compared the monosaccharides and molecular weight distributions of LLPs. Figure 1 shows that HPSEC-RID chromatograms of LLP-H, LLP-M, and LLP-V were similar, but LLP-RF and LLP-F were slightly different from them. Three distinct fractions were found in each sample, which characterized as polysaccharide fraction 1 ( $0.81\text{--}1.94 \times 10^5$  Da), polysaccharide fraction 2 ( $1.12\text{--}7.41 \times 10^4$  Da), and polysaccharide fraction 3 ( $5.17\text{--}60.09 \times 10^3$  Da). Results indicated that molecular weight distributions of LLPs varied by drying technologies. The detailed molecular weight distributions of polysaccharide fractions 1–3 in LLPs are summarized in Table 2 and are similar to the results of a previous study [4]. Molecular weights of LLP-H, LLP-M, and LLP-RF were obviously lower than that of LLP-F and LLP-V, because the increase of temperature might cause the decrease of molecular weights [22]. It is observed that polysaccharides with low molecular weight possess much higher antioxidant activities than that of polysaccharides with high molecular weight [25,26]. Furthermore, the polydispersities of fraction 1 ranged from 1.10 to 1.72, fraction 2 ranged from 1.06 to 1.42, and fraction 3 ranged from 1.01 to 1.05, consistent with HPSEC chromatograms.

In addition, Figure 2A shows that all LLPs were mainly composed of galactose (Gal), arabinose (Ara), galacturonic acid (GalA), rhamnose (Rha), glucose (Glc), mannose (Man), and glucuronic acid (GlcA), as well as minor xylose (Xyl). However, the molar ratios of LLPs varied by drying technologies (Table 2). The high molar ratio of GalA was observed in LLP-F, which was in accordance with the content of uronic acids (Table 1). Based on the molar ratios, Gal, GalA, and Ara were identified as the dominant monosaccharides in LLPs, consistent with a previous study [6]. Results further indicated that pectin-type polysaccharides existed in LLPs [15,16].

**Table 2.** Molecular weight ( $M_w$ ) and constituent monosaccharides of LLPs.

	LLP-H	LLP-M	LLP-V	LLP-RF	LLP-F
	$M_w$ (Da)				
Fraction 1 ( $\times 10^5$ )	0.83 ( $\pm 0.40\%$ )	0.81 ( $\pm 0.32\%$ )	1.45 ( $\pm 0.28\%$ )	0.83 ( $\pm 0.28\%$ )	1.94 ( $\pm 0.97\%$ )
Fraction 2 ( $\times 10^4$ )	1.12 ( $\pm 1.69\%$ )	1.67 ( $\pm 1.31\%$ )	2.15 ( $\pm 0.62\%$ )	1.49 ( $\pm 0.89\%$ )	7.41 ( $\pm 1.25\%$ )
Fraction 3 ( $\times 10^3$ )	5.17 ( $\pm 4.02\%$ )	7.92 ( $\pm 4.71\%$ )	18.21 ( $\pm 1.72\%$ )	6.87 ( $\pm 7.16\%$ )	60.09 ( $\pm 1.85\%$ )
	Monosaccharides and molar ratios				
Galactose	1.00	1.00	1.00	1.00	1.00
Galacturonic acid	0.69	0.69	0.60	0.82	1.02
Arabinose	0.74	0.78	0.68	0.76	0.82
Rhamnose	0.30	0.28	0.30	0.30	0.31
Glucose	0.33	0.31	0.25	0.32	0.37
Mannose	0.25	0.25	0.24	0.49	0.30
Glucuronic acid	0.19	0.17	0.17	0.16	0.20
Xylose	0.03	0.04	0.11	0.03	0.04

The sample codes were as the same in Table 1; Values represent mean ± standard deviation, and superscripts a-d differ significantly ( $p < 0.05$ ) among LLPs; Statistical significances were carried out by ANOVA plus post hoc Duncan's test.

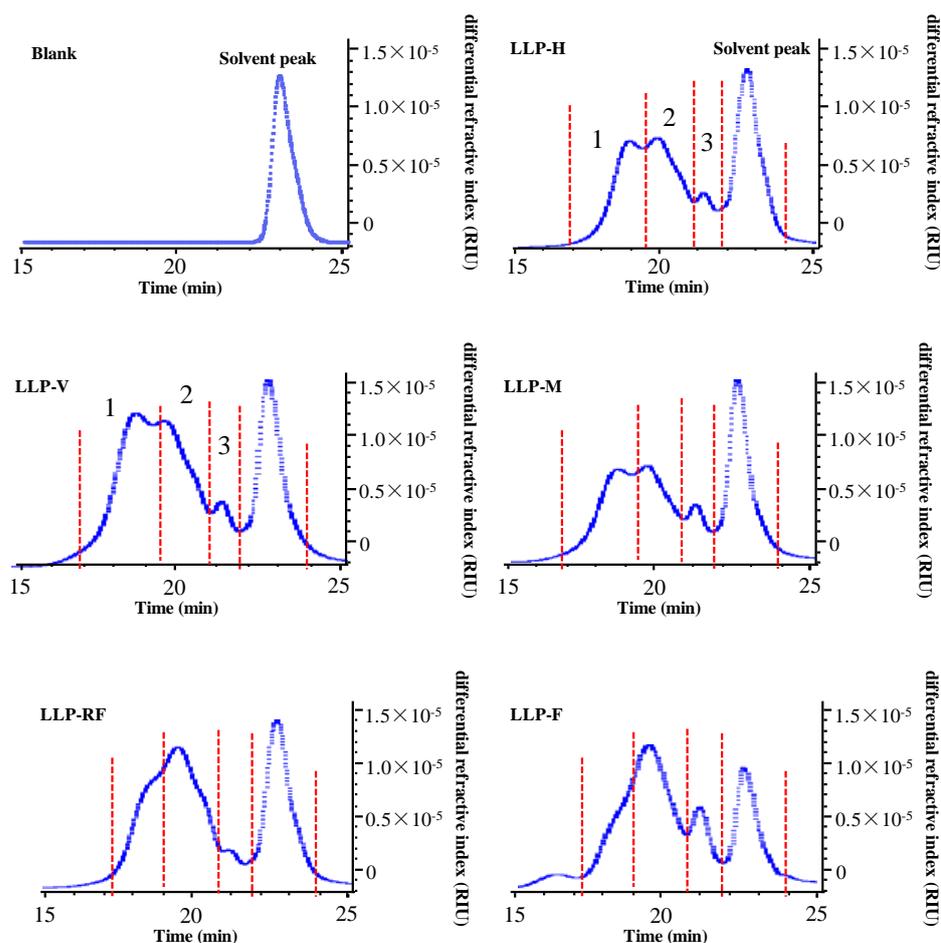


Figure 1. High-performance size-exclusion chromatograms of LLPs.

The sample codes were as the same in Table 1.

### 3.1.3. FT-IR and NMR Spectra of LLPs

FT-IR analysis showed that LLPs obtained by different drying technologies had similar IR absorption profiles (Figure 2B), indicating that LLPs possessed similar chemical groups. The strong absorption bands at the ranges of  $3600\text{--}3000\text{ cm}^{-1}$ ,  $3000\text{--}2800\text{ cm}^{-1}$ ,  $1700\text{--}1500\text{ cm}^{-1}$ ,  $1400\text{--}1200\text{ cm}^{-1}$ , and  $1200\text{--}700\text{ cm}^{-1}$  were typical bands of polysaccharides [4,6]. The absorption band at  $3432\text{ cm}^{-1}$  was responsible for the stretching vibration of -OH group [6], and the tensile vibration at  $2952\text{ cm}^{-1}$  was assigned to the signal of C-H [14]. The absorption band at  $1744\text{ cm}^{-1}$  suggested the stretching of the esterified carboxyl group, while the relatively strong absorption band at about  $1611\text{ cm}^{-1}$  indicated the existence of carboxyl or carbonyl groups in LLPs, further confirming that LLPs contained acidic polysaccharides [16]. The absorption band at  $1429\text{ cm}^{-1}$  was the feature of the C-H or the O-H [15]. Moreover, the absorption band around  $1240\text{ cm}^{-1}$  could be caused by the C-O-C of the methyl ester groups. In addition, absorption bands between  $1200\text{ cm}^{-1}$  and  $1000\text{ cm}^{-1}$  showed stretching vibrations of the C-O-H and the C-O-C, suggesting that the pyranose sugars existed in LLPs [6]. Moreover, the characteristic absorption band at  $890\text{ cm}^{-1}$  indicated the  $\beta$ -glycosidic linkages in LLPs [27]. Furthermore, the degrees of esterification (DE) of LLPs obtained by different drying technologies were also determined by FT-IR spectroscopy analysis. As shown in Table 1, the DE values of LLPs ranged from 13.34% to 22.34%. The highest DE value (22.34%) was found in LLP-F among LLPs, and the lowest DE value (13.34%) was found in LLP-H. Previous studies revealed that the lower DE value of natural polysaccharides might be closely associated with their higher antioxidant activity [16].

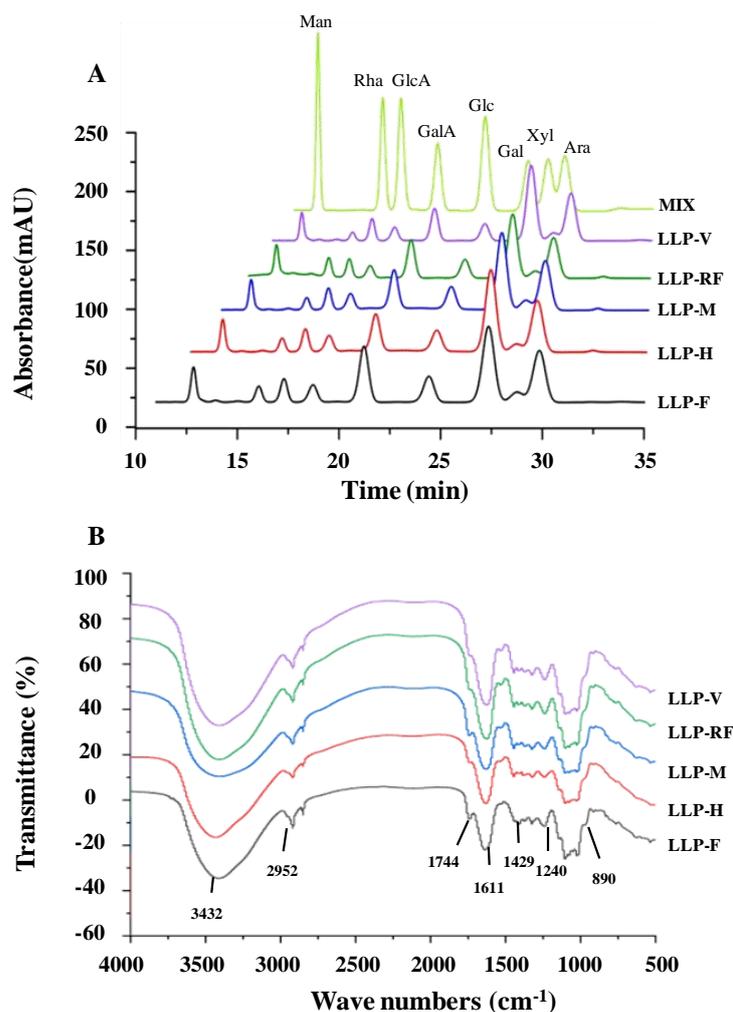


Figure 2. HPLC profiles of constituent monosaccharides (A) and FT-IR spectra (B) of LLPs.

The sample codes were as presented in Table 1.

In order to reveal the detailed structural features of LLPs obtained by different drying techniques, the NMR spectra were also recorded to analyze their structural information. As shown in Figures 3 and 4, the <sup>1</sup>H and <sup>13</sup>C NMR spectra of LLPs obtained by different drying technologies were similar, indicating that drying technologies did not change their backbones (main glycosidic linkages). The <sup>1</sup>H signal at 4.80 ppm was assigned to HOD. The signal at 4.98 ppm in the <sup>1</sup>H NMR spectra was assigned to the H-1 of 1, 4- $\alpha$ -D-GalA linkage, and its signals of C-1, C-2, C-3, and C-6 were 100.39 ppm, 68.07 ppm, 70.80 ppm, and 170.61 ppm, respectively [18,28]. A very intense signal at 3.82 ppm could be assigned to the methyl ester groups of the GalA carboxyl groups with the signal at 52.81 ppm in the <sup>13</sup>C NMR spectra [18]. Signals at 5.26, 3.73, and 1.26 ppm were attributed to the H-1, H-5, and H-6 of 1, 2- $\alpha$ -L-Rha linkage, respectively, and 81.29 ppm was assigned to the C-2 of 1, 2- $\alpha$ -L-Rha linkage [29]. The signal at 4.48 and 3.96 ppm belonged to the H-1 and H-6 of 1, 6- $\beta$ -D-Gal linkage [18,30]. The signal at 4.23 ppm was assigned to the H-4 of 1, 4- $\beta$ -D-Gal linkage, and the peaks at 73.18 and 78.93 ppm were connected to the C-2 and C-4 of 1, 4- $\beta$ -D-Gal linkage [18,31]. The peaks at 5.11 and 4.02 ppm suggested the H-1 and H-3 of 1, 5- $\alpha$ -L-Ara linkage, respectively, with signals at 109.16 and 83.79 ppm were derived from the C-1 and C-2 of 1, 5- $\alpha$ -L-Ara linkage [32], respectively. The signal at 4.16 ppm was derived from the H-2 of 1, 4- $\beta$ -D-Man linkage, while 76.74 and 60.77 ppm were corresponded to the C-4 and C-6 of 1, 4- $\beta$ -D-Man linkage [33]. The signals at 5.46 corresponded to the H-1 of 1,4- $\alpha$ -D-Glc linkage [18]. Finally, results suggested that rhamnogalacturonan I (RG

I), homogalacturonan (HG), and arabinogalactan might exist in LLPs according to their monosaccharides, FT-IR, and NMR spectra [34].

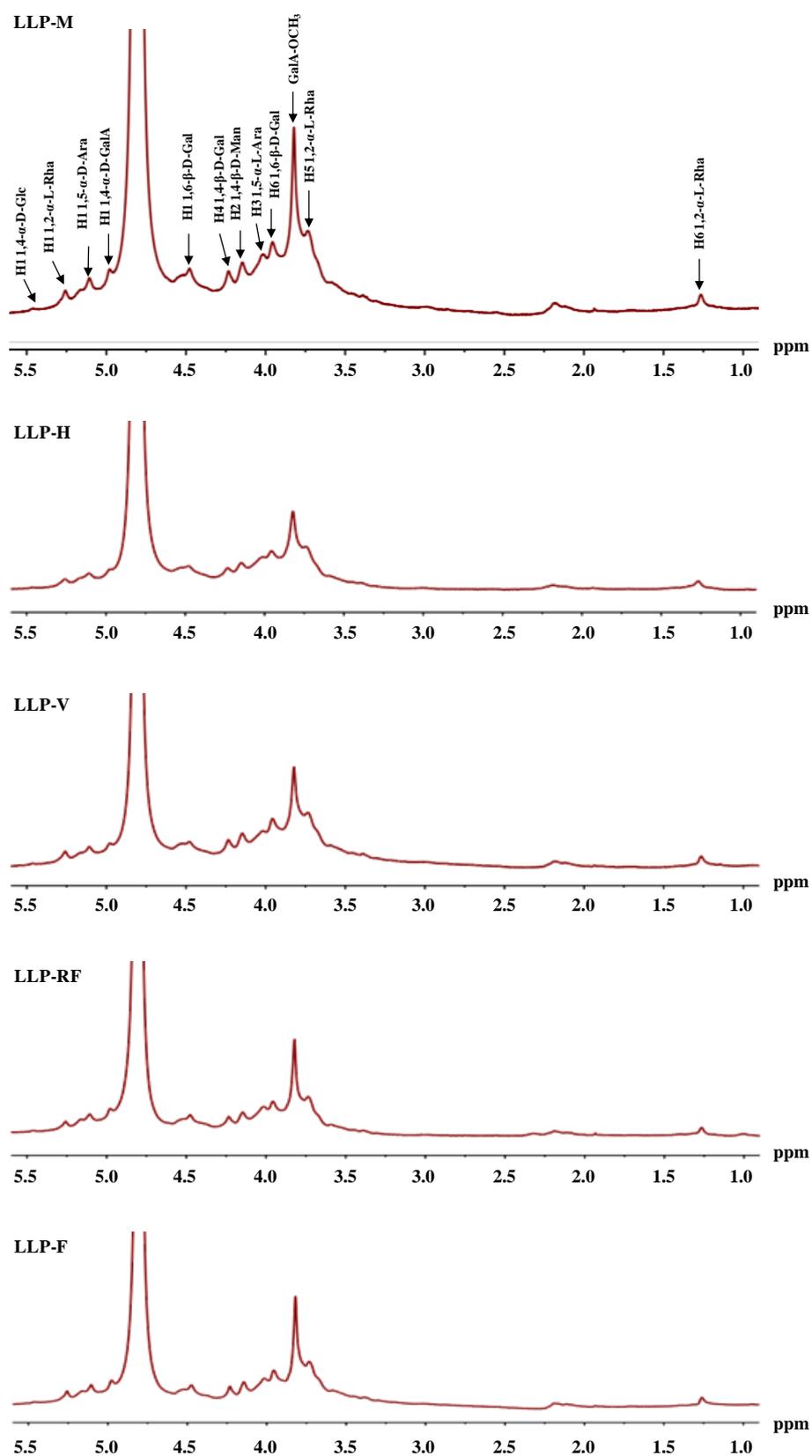


Figure 3.  $^1\text{H}$  NMR spectra of LLPs.

The sample codes were as the same in Table 1.

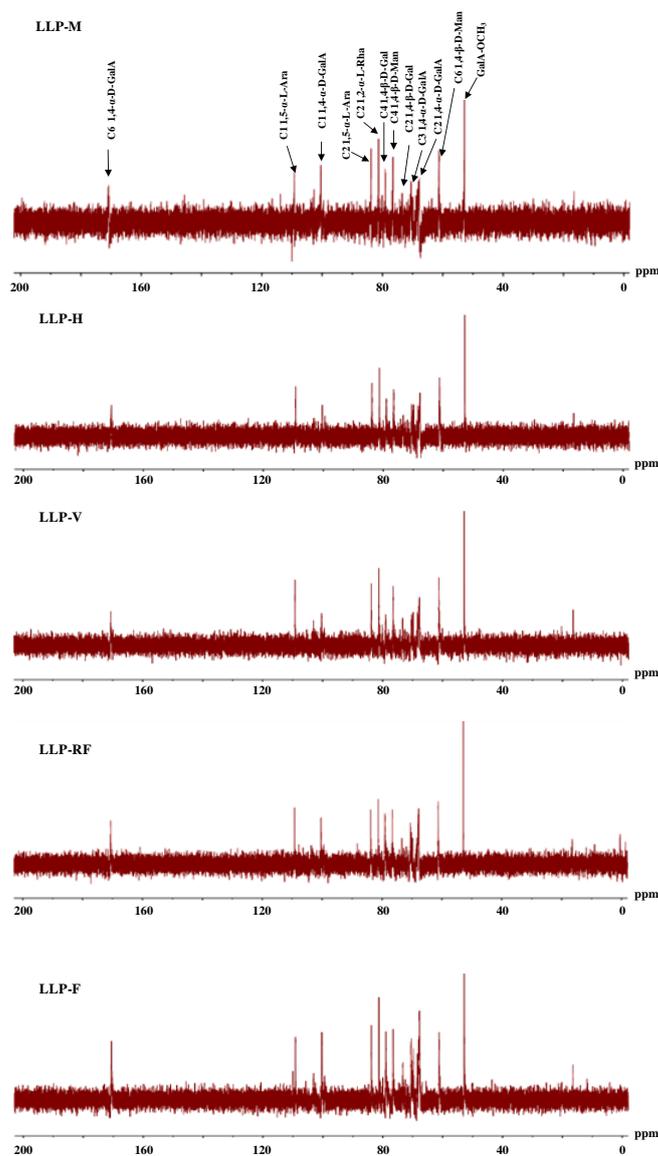


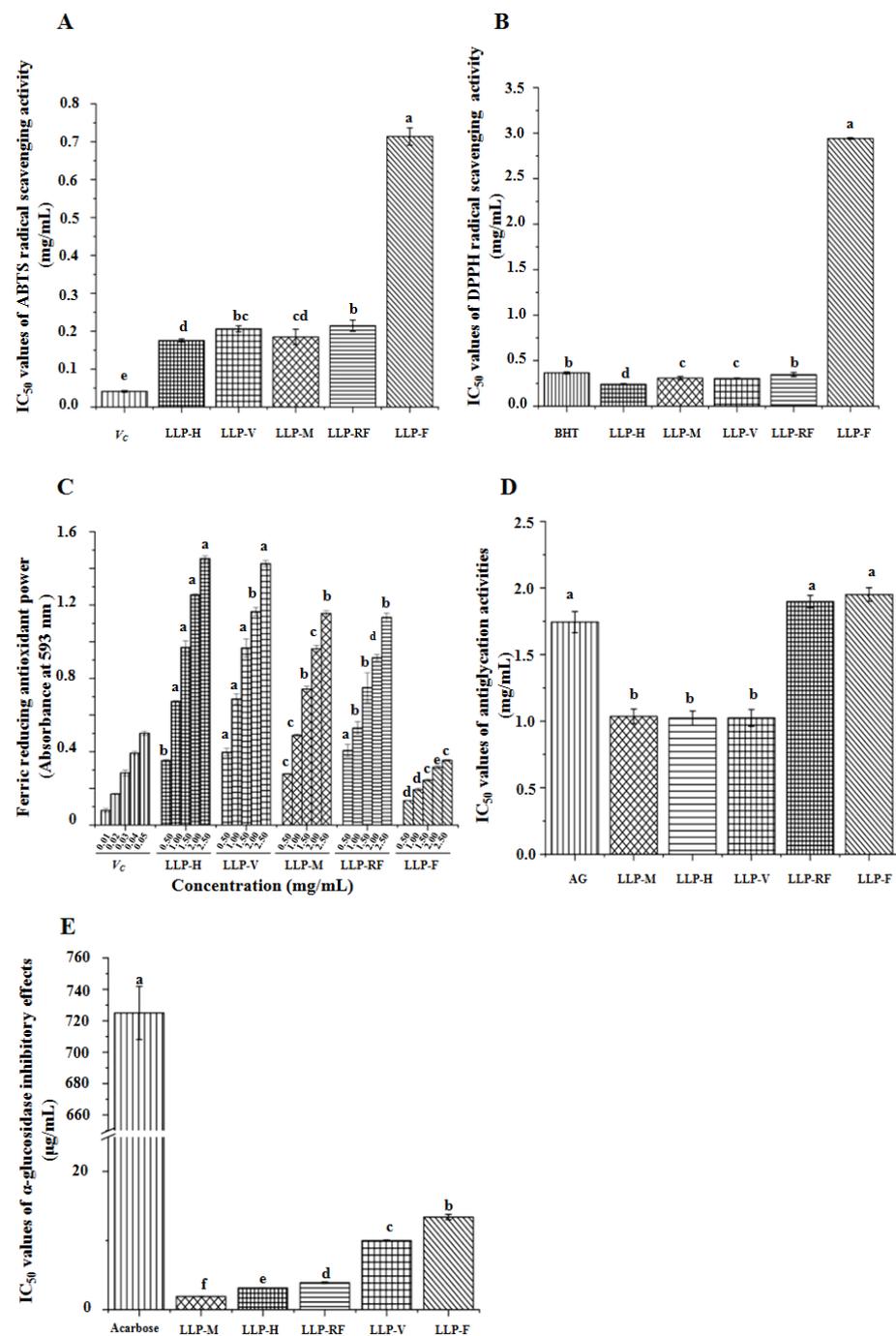
Figure 4.  $^{13}\text{C}$  NMR spectra of LLPs.

The sample codes were as the same in Table 1.

### 3.2. *In Vitro* Antioxidant Activities of LLPs Affected by Different Drying Techniques

In addition to the physicochemical properties, the biological properties of LLPs were further evaluated and compared. Several studies revealed that polysaccharides extracted from lotus leaves possessed significant antioxidant activities [4,6]. In this study, we compared the ABTS and DPPH radical scavenging activities, as well as the FRAP values of LLPs prepared by different drying techniques. As shown in Figure 5, LLPs obtained by different drying technologies exhibited potential antioxidant activities compared to the positive controls. Indeed, results indicated that antioxidant activities of LLPs varied by different drying technologies, which might be related to the different chemical properties affected by drying technologies. Briefly, the  $\text{IC}_{50}$  values of ABTS and DPPH radical scavenging activities of LLPs obtained by different drying techniques ranged from 0.176 mg/mL to 0.714 mg/mL, and from 0.241 mg/mL to 2.942 mg/mL, respectively. Moreover, the FRAP values of LLPs obtained by different drying techniques ranged from  $0.353 \pm 0.004$  to  $1.454 \pm 0.015$  at the concentration of 2.5 mg/mL. Results indicated that LLP-H obtained by

hot air drying exhibited higher ABST, DPPH, and FRAP antioxidant capacities than that of others, suggesting that the hot air drying technique might be a good method for drying lotus leaves to obtain polysaccharides with high antioxidant activities. In addition, compared with LLP-F, LLP-M, LLP-V, and LLP-RF also exhibited stronger antioxidant capacities.



**Figure 5.** ABTS (A), DPPH (B), and FRAP (C) antioxidant capacities, antiglycation activities (D), and inhibitory effects on  $\alpha$ -glucosidase (E) of LLPs.

Generally, a number of studies have demonstrated that the antioxidant activities of plant polysaccharides are connected to the chemical characteristics, molecular weight distributions, compositional monosaccharides (uronic acids), contents of proteins, degree of esterification, and bound polyphenolics [14,16,35]. Polysaccharides with lower molecular weight distributions exhibit higher antioxidant activities [21,22], which may be due

to their more loose and porous structures, resulting in more reductive hydroxyl groups to react with free radicals. Indeed, the contents of free uronic acids can also contribute to the antioxidant activities of polysaccharides due to the electrophilic groups of acidic polysaccharides, which can facilitate the liberation of hydrogens. Moreover, the low DE value of natural polysaccharides could contribute to their relatively high antioxidant activity [16]. Furthermore, the bound polyphenolics can also enhance the biological activities of natural polysaccharides [19,24]. In the present study, compared with LLP-F, the higher antioxidant capacities observed in LLP-H, LLP-M, LLP-V, and LLP-RF might be due to the combination effect of low molecular weight, high content of free uronic acids, and high content of bound polyphenolics [14,18,21]. However, further studies are required to reveal the detailed structure-antioxidant activity of LLPs in the future.

The sample codes were as the same in Table 1. BHT, butylated hydroxytoluene; Vc, vitamin C; AG, aminoguanidine; The error bars are standard deviations; Significant ( $p < 0.05$ ) differences are shown by data bearing different letters (a–e); Statistical significances were carried out by ANOVA and post hoc Duncan's test.

### 3.3. *In Vitro* Antiglycation Activities of LLPs Affected by Different Drying Techniques

Advanced glycosylation end products (AGEs) can lead to several chronic diseases, such as arteriosclerosis, aging, and diabetes complications, which are produced by the spontaneous non-enzymatic aminocarbonyl reaction between reducing sugars and proteins [14]. In this study, we compared the antiglycation activities of LLPs obtained by different drying technologies. Figure 5D shows that LLPs possessed stronger antiglycation activities than that of AG ( $IC_{50} = 1.744 \pm 0.080$  mg/mL). The antiglycation activities of LLPs also varied by different drying technologies, and their  $IC_{50}$  values ranged from 1.023 to 1.951 mg/mL. Results revealed that LLP-M, LLP-H, and LLP-V exerted stronger antiglycation activities than LLP-RF and LLP-F. Previous studies suggested that the antiglycation effect might be associated with the antioxidant activity [14,36], and the high content of bound polyphenolics also contributed to their antiglycation effect.

### 3.4. *In Vitro* $\alpha$ -Glucosidase Inhibitory Effects of LLPs Affected by Different Drying Techniques

The  $\alpha$ -glucosidase inhibitory activity can combat the metabolic changes associated with type 2 diabetes, and it is also one of the major strategies for treatment of type 2 diabetes [37]. Some studies revealed that pectin-type polysaccharides possessed anti-diabetic activities [21,38], and polysaccharides from lotus leaves also exerted anti-diabetic effect [7]. Therefore, we compared the *in vitro*  $\alpha$ -glucosidase inhibitory activities of LLPs obtained by different drying technologies (Figure 5E). Potent inhibitory effects against  $\alpha$ -glucosidase of LLPs were observed, which also varied by different drying technologies. The  $IC_{50}$  values of LLPs ranged from  $1.90 \pm 0.02$  to  $13.40 \pm 0.38$   $\mu$ g/mL, which were significantly lower than that of acarbose ( $IC_{50} = 724.98 \pm 16.93$   $\mu$ g/mL). The results revealed that LLP-M prepared by microwave drying exhibited the highest  $\alpha$ -glucosidase inhibitory activity among LLPs, while the weakest  $\alpha$ -glucosidase inhibitory effect was observed in LLP-F among LLPs. In addition, the  $\alpha$ -glucosidase inhibitory of LLPs was higher than that of polysaccharides obtained from dandelion [18] and okra [16].

Generally, polysaccharides can bind with the free  $\alpha$ -glucosidase to change its enzyme structure, decreasing its catalytic abilities [39,40]. At the same time, polysaccharides can also restrain the digestion of substrate by binding to the substrate and enzyme to form a ternary complex [39]. Usually, the inhibitory effects of natural polysaccharides on  $\alpha$ -glucosidase are associated with their chemical properties, such as molecular weights, monosaccharide compositions, contents of free uronic acids, and contents of bound polyphenolics. Studies have reported that polysaccharides with lower molecular weights from *Zingiber officinale* and blackberry fruit exhibit higher inhibitory activities on digestive enzymes [39,40]. Additionally, the high degree of esterification of pectic-polysaccharides might contribute to their high inhibitory effects on digestive enzymes [41]. Furthermore, the high content of bound polyphenolics may also contribute to the high

inhibitory effects of natural polysaccharides against digestive enzymes [14,18,42]. Therefore, in the present study, the lower molecular weight distribution, higher degree of esterification, and higher content of bound polyphenolics of LLP-M might contribute to its stronger inhibitory effect on  $\alpha$ -glucosidase. However, besides the chemical components determined in LLPs (Table 1), other phytochemicals might also exist in LLPs. Therefore, further studies are required to well reveal the potential structure–function relationships of LLPs in the future.

#### 4. Conclusions

In this study, the physicochemical and biological properties of polysaccharides from lotus leaves obtained by different drying technologies were systematically evaluated. The results revealed that drying techniques could alter the physicochemical characteristics and biological activities of lotus leaf polysaccharides. The low molecular weight distributions were observed in LLP-H, LLP-M, and LLP-RE, respectively. The high contents of bound polyphenolics were measured in LLP-H, LLP-M, and LLP-V, respectively. Besides, all LLPs exhibited obvious in vitro antioxidant capacities, antiglycation activities, and inhibitory effects on  $\alpha$ -glucosidase. LLP-H exhibited significant higher antioxidant activities than others, and LLP-M exerted stronger antiglycation and inhibitory effects on  $\alpha$ -glucosidase than others. The findings from this study could provide fundamental knowledge for selecting suitable drying methods for the pre-processing of lotus leaves.

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