Comparison of Physicochemical and Immunomodulatory Properties of Polysaccharides from Fresh and Dried Longan Pulp Products

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Abstract: Longan is a popular fruit and is widely distributed throughout the subtropical area. The dried pulp of longan has been used in traditional Chinese medicine and is made up of polysaccharides as one of the main bioactive ingredients. The nutritional difference between fresh and dried longan pulp products was investigated using analytical methods and the physicochemical properties and immunomodulatory activity of polysaccharides were evaluated. Gas chromatography-mass spectrometry (GC-MS), ion exchange chromatography, gel filtration chromatography, viscometry, ultraviolet (UV) spectroscopy, and infrared (IR) spectroscopy were performed. The results showed that the longan polysaccharides from fresh and dried fruits (i.e., LPF and LPD, respectively) were polysaccharide-protein complexes, but significant differences were found in their binding protein contents, neutral/acid polysaccharide ratios, monosaccharide compositions, molecular weight distributions, and intrinsic viscosities. Cellular *in vitro* tests confirmed LPD strongly stimulated lymphocyte proliferation and macrophage phagocytosis compared to LPF in the dose range of 50~400 μg/mL. Both polysaccharides significantly stimulated ConA-induced lymphocyte proliferation and nitric oxide production from macrophages ($P < 0.05$), but had a weak effect on lipopolysaccharide (LPS)-induced lymphocyte proliferation. The drying process of longan pulp may enhance the *in vitro* immunomodulatory activity of water-soluble polysaccharides by changing their physicochemical characteristics.

Key words: longan pulp; polysaccharides; physicochemical property; immunomodulatory activity; drying

Article No.: 1673-9078(2015)10-53-62 DOI: 10.13982/j.mfst.1673-9078.2015.10.010

新鲜和干制龙眼果肉中多糖的理化特征及免疫调节活性比较

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摘要： 龙眼是一种颇受欢迎的亚热带水果，其干制果肉作为传统中药材亦被人们所熟知，而多糖是主要的生物活性成分。基于多糖理化特征和免疫调节活性的分析评价，探析新鲜和干制龙眼果肉的营养性差异。通过气质联用、离子交换层析、凝胶过滤层析、粘度测定、紫外和红外光谱分析发现，新鲜果肉多糖（LPF）和干制果肉多糖（LPD）均为多糖-蛋白络合物，但两者的结合蛋白含量、中性与酸性多糖含量比、单糖组成、分子量分布和特征粘度均存在显著差异。体外细胞试验证实，LPD 在 50–400 μg/mL 剂量范围内对脾淋巴细胞增殖和巨噬细胞吞噬的增强作用明显强于 LPF。LPD 和 LPF 均能显著刺激 ConA 诱导的脾淋巴细胞增殖和巨噬细胞 NO 生成($P<0.05$)，但对 LPS 诱导的脾淋巴细胞增殖刺激作用较弱。龙眼果肉的干制处理可能通过改变水溶性多糖的理化特征而增强其体外免疫调节活性。

关键词： 龙眼果肉；多糖；理化特征；免疫调节活性；干制

收稿日期：2014-12-30

基金项目：国家自然科学基金资助项目（31301416）

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Longan (*Dimocarpus longan* Lour.) is a subtropical evergreen tree of the *Sapindaceae* family, its fruit is rich in polysaccharides, proteins, fibers, vitamin C, amino acids, and minerals [1]. The longan fruit is seasonal and is not cultivated on a large scale due to its perishable nature, short shelf-life,

and susceptibility to postharvest diseases [2]. In China, almost 70% of longan fruits are marketed fresh during August and September, and the remaining fruits are mainly consumed in the dehydrated forms [3]. Dried longan pulp has been traditionally used in Chinese medicinal formulations, and aids in the improvement of blood metabolism, alleviation of neural pain, insomnia, and amnesia [4]; however, the fresh pulp does not exhibit these characteristics. We are yet to ascertain whether the longan pulp should be consumed in dried form to maximize its biological benefits. It is still unknown whether the bioactivity of dried longan pulp is better than that of fresh pulp.

Immunodeficiency occurs when the immune system is compromised rather than when it is in the normal state, thereby resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be caused by genetic diseases or acquired conditions, such as the use of immunosuppressive medications. Both dried longan pulp and its aqueous extract can significantly strengthen the immune system of normal mice [5]. Furthermore, our previous works have confirmed that main active components contributing to the immunomodulatory function of dried longan pulp are polysaccharides, which can effectively enhance specific and nonspecific immunity in mice that are immunosuppressed with cyclophosphamide [4,6]. It is known that the bioactivities of native polysaccharides are closely related to their chemical composition and structure, which are sensitive to thermal treatment. For example, the conformation of lentinan irreversibly transforms from a triple strand to a winding chain rapidly at 130~145 ℃ [7], resulting in a significant decrease in anti-tumor activity [8]. Similarly, thermal drying could modify the structures of *Inonotus obliquus* polysaccharides and cause aggregation by partial removal of the hydration layer, producing polysaccharides with higher molecular weight and weaker antioxidant activity than those subjected to freeze drying [9]. In comparison with the crude polysaccharides obtained from fresh fruit of *Lentinus polychrous* Lév, those obtained from the dried fruit have higher protein content, better antioxidant activity, and stronger cytotoxic effect against MCF-7 tumor cells [10]. However, the structural and immunomodulatory differences between polysaccharides from fresh and dried longan pulp, which are important to reveal the effect of the drying process on the health benefits of the pulp, still remain unclear.

The aim of this study was to investigate the physicochemical properties and immunomodulatory activities of polysaccharides from fresh and dried longan pulp. Their chemical compositions, molecular weight distributions, intrinsic viscosities, ultraviolet (UV), and fourier transform infrared (FT-IR) spectra were analyzed. In addition, their effects on the proliferation of splenic lymphocytes, phagocytosis, and nitric oxide (NO) production in macrophage were also further evaluated *in vitro*.

1 Materials and methods

1.1 Plant materials

Fresh longan fruits (*cv*. Chu-liang) in the maturation stage were provided by Pomology Research Institute of Guangdong Academy of Agricultural Sciences (Guangzhou, China). Fruits, uniform in size and color were selected and parts of these fruits were then dried in hot air at 75 ℃ for 50 h.

1.2 Preparation of longan pulp polysaccharides

Longan pulp (200 g of fresh or 50 g of dried pulp) was extracted with 1 L distilled water under constant stirring speed of 120 r/min and at a temperature of 80 ℃ for 4 h. After centrifugation at 4,500 r/min for 10 min followed by filtration through a Whatman No. 1 paper, the extract was concentrated at 55 ℃ using a vacuum rotary evaporator (Eyela, Japan). The concentrate was dialyzed against distilled water at 4 ℃ for 72 h to remove the micromolecular substances. The dialyzed solution was concentrated again and then freeze-dried to produce polysaccharide powder. The yield of the polysaccharides from fresh longan pulp was 33.4 g/kg and that from dried longan pulp was 120.1 g/kg.

1.3 Physicochemical analysis

1.3.1 Chemical composition analysis

The polysaccharide content was determined by phenol-sulfuric acid method [11] and expressed as glucose equivalents. A GC-MS method described in our previous study was adopted for the identification and quantification of monosaccharides in a polysaccharide sample [4].

Hydrochloric acid solution (15 mL, 6 mol/L) and redistilled phenol (4 drops) were added in a hydrolysis tube containing 150~200 mg sample. The tube was fully sealed with N_2 and kept at 110 ℃ in an oven for 22 h. Subsequently, the hydrolyzate was diluted with distilled water to a constant volume of 50 mL. The diluted

hydrolyzate (1 mL) and 4 mL distilled water were added to a 25 mL beaker and vacuum-dried at 50 ℃. The residue was dissolved in 2 mL distilled water, vacuum-dried, and then dissolved in 1 mL sodium citrate buffer (pH 2.2). The solutions (20 μL) were finally analyzed using an automatic amino acid analyzer (Hitachi L-8900, Japan).

Neutral and acid polysaccharides in the sample were analyzed using ion exchange chromatography [4]. The sample (10 mg) was dissolved in 2 mL distilled water. After centrifugation at 4,500 r/min for 15 min, 1 mL of supernatant was injected onto a DEAE52-cellulose column (20 cm × 1.5 cm, filler was purchased from Sigma) equilibrated with distilled water. The column was eluted with distilled water for 10 h and NaOH solution (0.5 mol/L) for 10 h. The eluant (2.4 mL/tube) was collected at a flow rate of 0.16 mL/min. The concentrations of the polysaccharides and proteins in the eluant were determined by the phenol-sulfuric acid method and UV method, respectively, and expressed as the optical density (*OD*) value. The plot of the *OD* value *vs.* elution volume was then established.

1.3.2 Molecular weight distribution analysis

The molecular weight distribution of the polysaccharides was analyzed by gel filtration chromatography [12]. The sample (10 mg) was dissolved in 2 mL distilled water. After centrifugation at 4500 r/min for 15 min, 1 mL supernatant was injected into a Sephadex G-100 gel column (20 cm × 1.5 cm, filler was purchased from Sigma) followed by elution with distilled water at a flow rate of 0.1 mL/min. The eluant (1.5 mL/tube) was collected and assayed using phenol-sulfuric acid method and UV method. The plot of *OD* value *vs*. elution volume was then established.

1.3.3 Intrinsic viscosity measurement

The intrinsic viscosity (η) of the polysaccharides in water was measured at 25 ±0.05 ℃ using an Ubbelohde capillary viscometer (0.46 mm) [13]. The sample solution (10 mL, 20 mg/mL) was filtered through a fritted glass Buchner funnel (15~40 μm). Filtered solution (7 mL) was used to measure the reduced viscosity (η_{sp}/c). Data obtained was the average of the three measurements. The kinetic energy correction was negligible. η_{sp}/c was defined by Huggins equation:

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad (1)$$

where *k'* is a constant and *c* is the polysaccharide concentration. It can be used to estimate the (η) value by extrapolating to infinite dilution.

1.3.4 UV and IR spectra analysis

The sample (5 mg) was dissolved in 5 mL distilled water. After centrifugation at 4500 r/min for 15 min, 2 mL supernatant was added into a tube containing 2 mL distilled water or 2 mL NaOH solution (0.4 mol/L) followed by incubation at 45 ℃ for 1.5 h. The UV spectrum of the mixture was then scanned at a wavelength range of 190~400 nm using a UV1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan) [12]. The FT-IR spectrum of the sample was determined using a Fourier transform infrared spectrophotometer (Nexus 5DXC FT-IR, Thermo Nicolet, America). The sample was ground with a spectroscopic grade potassium bromide powder and then pressed into a 1-mm pellet for measurement in the frequency range of 4,000~400 cm^{-1}.

1.4 Immunomodulatory evaluation

1.4.1 Lymphocyte proliferation analysis

Ten-week-old BALB/c mice (specific pathogen-free, male) were sacrificed by cervical dislocation, and their spleens were aseptically dissected to harvest lymphocytes [4]. The cell proliferation stimulated by polysaccharides was analyzed by the MTT method described by Li et al. [14]. The test was approved by the Laboratory Animal Committee of Guangdong Province, and the animals were treated according to the Guide for the Care and Use of Laboratory Animals. Splenocytes were suspended in RPMI 1640 complete medium (10% fetal bovine serum, Gibco BRL, Grand Island, NY, USA) and adjusted to a concentration of 1 × 10^7 cells/mL. The suspension (50 μL/well) was plated in a 96-well culture plate with or without mitogen (5.0 μg/mL ConA or LPS, Sigma, St. Louis, MO, USA). The polysaccharide sample was dissolved using complete medium, filter-sterilized, and then added into the cell well at various final concentrations (0, 50, 100, 200, and 400 μg/mL). After incubation for 68 h (37 ℃, 5% CO_2), each well was pulsed with 10 μL MTT (5 mg/mL, Sigma). The plate was further incubated for 4 h. Acidified isopropyl alcohol (100 μL/well) was then added to dissolve the formazan

crystals for 12 h. The plate was finally analyzed at 570 nm using a microplate reader (Thermo Labsytems, Helsinki, Finland). The stimulation index (*SI*, %) of lymphocyte proliferation was calculated based on the following equation:

$$SI = (OD_S - OD_C) / ODC \times 100 \quad (2)$$

where OD_c and OD_s represent the *OD* values of the control and stimulated group, respectively.

1.4.2 Macrophage phagocytosis analysis

RAW264.7 macrophage cell line was provided by the Experiment Animal Center of Sun Yat-sen University (Guangdong, China). After adjusting the cell concentration to 5 × 10^5 cells/mL, 100 μL/well of the macrophage suspension was plated in a 96-well culture plate and incubated for 3 h (37 ℃, 5% CO_2). The macrophages were washed twice with DMEM complete medium (containing 10% fetal bovine serum, Gibco, USA) and incubated with 100 μL/well of stimulant (final polysaccharide concentrations, 0, 50, 100, 200, or 400 μg/mL; LPS, 5 μg/mL) for another 24 h. The cells were washed twice with phosphate buffered saline (PBS) and 0.1% neutral red (100 μL/well) was then added in. The plate was further incubated for 4 h. After washing out non-phagocytized neutral red with PBS, 100 μL/well of cell lysis solution (the volume ratio of acetic acid to ethanol was 1:1) was added and kept for 12 h. The *OD* value of each well was read at 570 nm using a microplate reader. The phagocytosis index (*PI*, %) of the macrophage against neutral red was calculated based on the equation:

$$PI = (OD_S - OD_C) / OD_C \times 100 \quad (3)$$

where OD_S represents the *OD* value of stimulated group, and OD_C represents that of the control [15].

1.4.3 Macrophage nitric oxide production analysis

Nitrite accumulation was used as an indicator of nitric oxide (NO) production in culture medium based on the Griess reaction [16]. A macrophage suspension (5 × 10^5 cells/mL) was plated in a 24-well culture plate (500 μL/well) and incubated for 3 h (37 ℃, 5% CO_2). After washing the cells that had not adhered, 500 μL/well of stimulant (final concentration of polysaccharide: 0, 50, 100, 200, or 400 μg/mL; LPS, 5 μg/mL) was added and incubated for an additional 24 h. The culture medium (150 μL) from each well was collected in 1.5-mL centrifuge tubes containing 10 μL $ZnSO_4$ solution (300 mg/mL). The mixture was centrifuged at 4,500 r/min for 10 min. The supernatant (100 μL) was then mixed with 100 μL Griess reagent in a 96-well culture plate for 10 min at room temperature and then analyzed at 540 nm. The concentration of NO released by macrophages was calculated according to the standard curve established by $NaNO_2$. The NO production index (*NI*, %) was expressed as the following equation:

$$NI = (C_S - C_C) / C_C \times 100 \quad (4)$$

where C_S and C_C represented the NO concentration of stimulated group and control, respectively.

1.5 Statistical analysis

The data were expressed as means ± standard deviation. The significance in the difference was evaluated with one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test using IBM SPSS Statistics 19 software. A *P* value of 0.05 was used as the threshold for significance.

2 Results and discussion

2.1 Chemical composition of longan polysaccharides

The chemical compositions of LPD and LPF were different as shown in Table 1. The LPD had a higher protein content than LPF. The carbohydrate to protein ratios of LPD and LPF were 14.46 and 24.19, respectively. LPD was mainly composed of glucose, mannose, and arabinose in the molar ratios of 5.71:1.02:1.00, while LPF was mainly composed of glucose and mannose in the molar ratio of 5.19:1. In addition, seventeen amino acids were detected in the samples, and the content of glutamic acid was the highest. Longan pulp polysaccharides from different cultivars showed significant differences in monosaccharide composition. Three polysaccharide of the 'Fenglisui' longan pulp were isolated and characterized to be β-type heteropolysaccharides with the pyran group, among which LPS-N consisted of xylose and glucose; LPS-A1 consisted of rhamnose, xylose, arabinose, and galactose, and LPS-A2 comprised only of rhamnose[17]. A novel polysaccharide isolated from the 'Shixia' longan pulp was identified to be a (1→6)-α-D-glucan composed of 661 glucose residuals[18].

Table 1 Chemical compositions of longan polysaccharide LPD and LPF

Component content /%	LPD	LPF
Protein	6.41±0.27	3.90±0.22
Polysaccharide	92.70±3.14	94.33±3.75
Monosaccharides		
Rhamnose	0.33±0.02	0.33±0.02
Arabinose	11.07±0.21	2.99±0.07
Xylose	0.72±0.03	0.83±0.03
Mannose	11.35±0.30	14.06±0.35
Glucose	63.22±1.41	73.02±1.78
Galactose	5.41±0.11	2.85±0.06
Amino acids		
Aspartate	0.45±0.02	0.34±0.01
Threonine	0.44±0.02	0.19±0.01
Serine	0.23±0.01	0.12±0.00
Glutamic	1.91±0.07	1.27±0.04
Glycine	0.24±0.01	0.17±0.01
Alanine	0.55±0.02	0.40±0.01
Cystine	0.07±0.00	0.04±0.00
Valine	0.41±0.02	0.16±0.01
Metione	0.08±0.00	0.08±0.00
Isoleucine	0.12±0.00	0.08±0.00
Leucine	0.24±0.01	0.12±0.00
Tyrosine	0.10±0.00	0.07±0.00
Phenylalanine	0.17±0.00	0.13±0.00
Lysine	0.56±0.03	0.21±0.01
Histidine	0.12±0.00	0.07±0.00
Arginine	0.16±0.01	0.07±0.00
Proline	0.36±0.02	0.21±0.01

There are two main polysaccharide peaks in both the DEAE-52 ion exchange chromatograms of LPD and LPF, as seen in Fig.1. The fraction eluted by distilled water belonged to the neutral polysaccharide and that eluted by 0.5 mol/L NaOH solution was an acidic polysaccharide. The peak area ratios of the acidic polysaccharide to the neutral polysaccharide of LPD and LPF were 4.35 and 1.82, respectively, thereby indicating higher acidic polysaccharide content in LPD. The protein response of LPD was obviously stronger than that of LPF. Moreover, the peaks of the acidic polysaccharide and protein responded consistently, thereby indicating the existence of binding proteins in both LPD and LPF.

2.2 Molecular weight distributions of longan polysaccharides

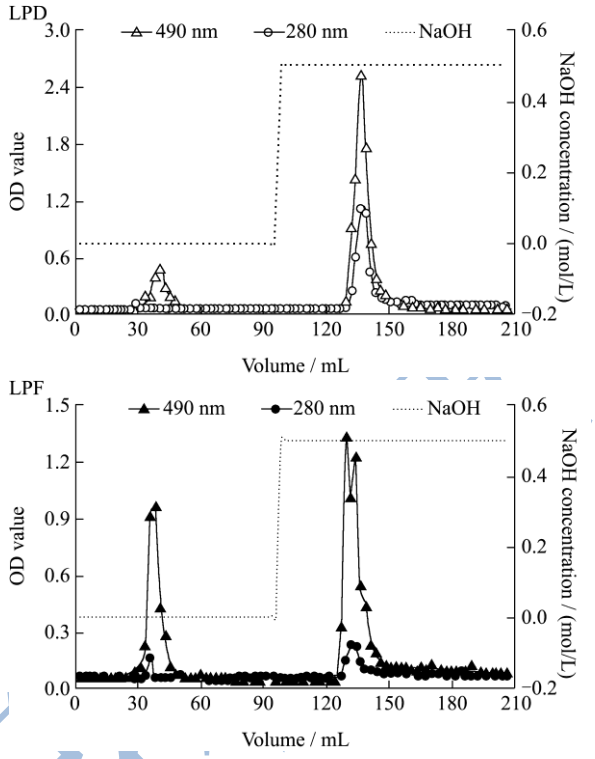

Fig. 1 DEAE-52 ion exchange chromatograms of longan polysaccharides LPD and LPF

Note: Polysaccharides and proteins were detected at 490 nm and at 280 nm, respectively.

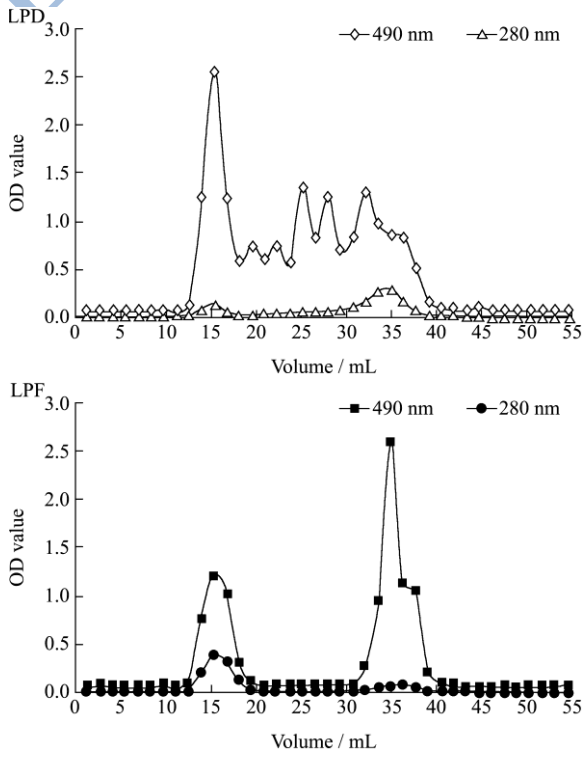

Fig. 2 Sephadex G-100 gel filtration chromatograms of longan polysaccharides (LPD and LPF)

Note: Polysaccharides and proteins were detected at 490 nm and at 280 nm, respectively.

As seen in Fig.2, the gel filtration chromatograms of LPD and LPF are significantly different. LPD had multiple polysaccharide peaks in the elution volume range of 12~40 mL, and its low molecular weight fraction possessed the highest protein binding ability. In contrast, two main polysaccharide peaks of LPF were symmetrically distributed at the peak value of 16 mL and 36 mL, respectively. The average molecular weight of LPF was less than that of LPD. Moreover, the proteins mainly bound to large molecular weight fractions in LPF. The results based on the chromatograms of the ion exchange column and gel column both indicated that LPD and LPF were polysaccharide-protein complexes.

2.3 Intrinsic viscosities of longan polysaccharides

The η_{sp}/c value of LPD is obviously smaller than that of LPF at any given sample concentration as shown in Fig.3. By extrapolating the linear plot of η_{sp}/c vs. the concentrations to infinite dilution, the molecular weight depended on the intrinsic viscosities of LPD and LPF in water, which were found to be 17.49 and 27.29 mL/g, respectively.

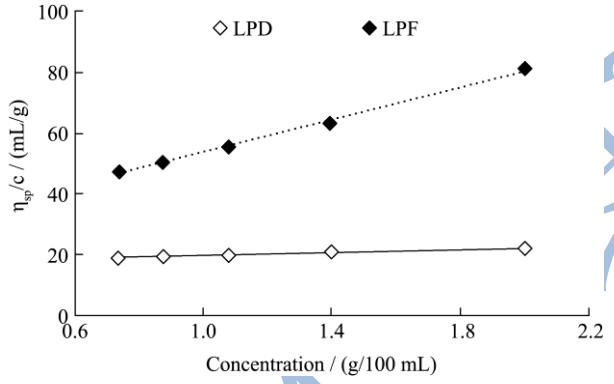

Fig. 3 Huggins' plots of longan polysaccharides (LPD and LPF) in water at 25 ℃

2.4 UV and FTIR spectra of longan polysaccharides

In Fig.4, the UV absorption at 280 nm indicated the presence of a few proteins in LPD, but the response was not observed for LPF. The β-elimination reaction was used for the detection of O-glycosidic bond in the polysaccharide-protein complex. After treatment with NaOH solution, obvious absorption peaks at 240 nm and 280 nm were found in the spectrum of LPD, and enhanced absorption at 240 nm was also observed in that of LPF, thereby indicating the existence of O-glycosidic bonds in both of them.

As seen in Fig.5, both LPD and LPF exhibited characteristic polysaccharide bands, which were mainly produced from hydroxyl groups (3,420 and 1,015 cm^{-1}), alkyl groups (2,935 and 1,458 cm^{-1}), and carboxyl groups (1,425, 1,365, and 1,265 cm^{-1}). In addition, the amino group bands (1,640 cm^{-1}) indicated the presence of proteins, while the fingerprint region absorptions (920, 865, 817, and 777 cm^{-1}) implied the main composition of α-D-glucose in LPD and LPF.

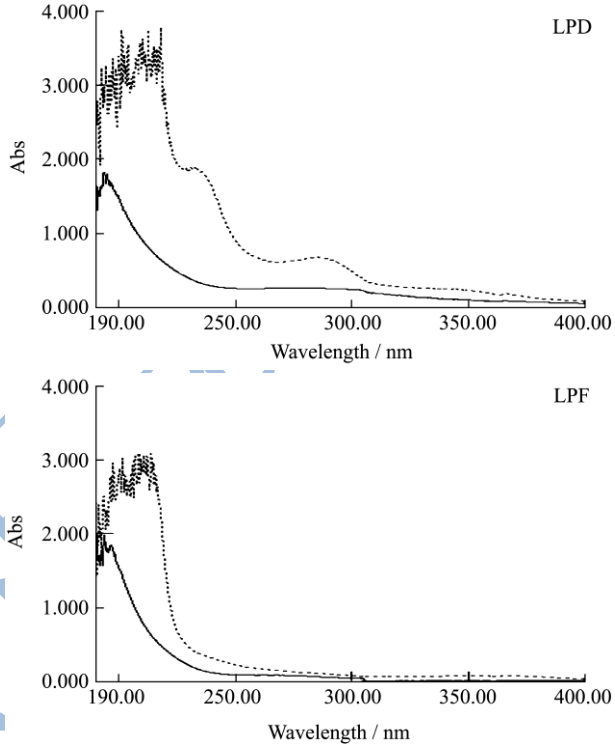

Fig. 4 UV spectra of β-elimination reactions of longan polysaccharides LPD and LPF

Note: The dash curve represented the absorption of polysaccharides treated with sodium hydroxide, and the solid curve represented that of the non-treated sample.

The physicochemical properties of LPD and LPF exhibited significant differences related to the thermal drying process of longan pulp. LPD had more binding proteins than LPF; this suggested that thermal effect induced conformational changes of polysaccharides and proteins forming protein-polysaccharide complexes by electrostatic interactions[19,20]. Moreover, the monosaccharide compositions of LPD and LPF were obviously different, and the same phenomenon was also observed in polysaccharides from fresh and sun-dried pear pulp [21]. Thermal processing may cause structural changes in the polysaccharides as well as aggregation of the low molecular weight fraction. For LPD and LPF, good linear

relationships between reduced viscosity and concentration resulted in normal solution behavior of the polysaccharide (Fig.3), but not the polyelectrolyte behavior [22,23]. As a sensitive parameter related to the extension of polymer chain, the (η) value revealed that the shape of the LPD molecule was relatively compact compared with LPF [22,24], thereby supporting the effect of thermally induced molecular aggregation.

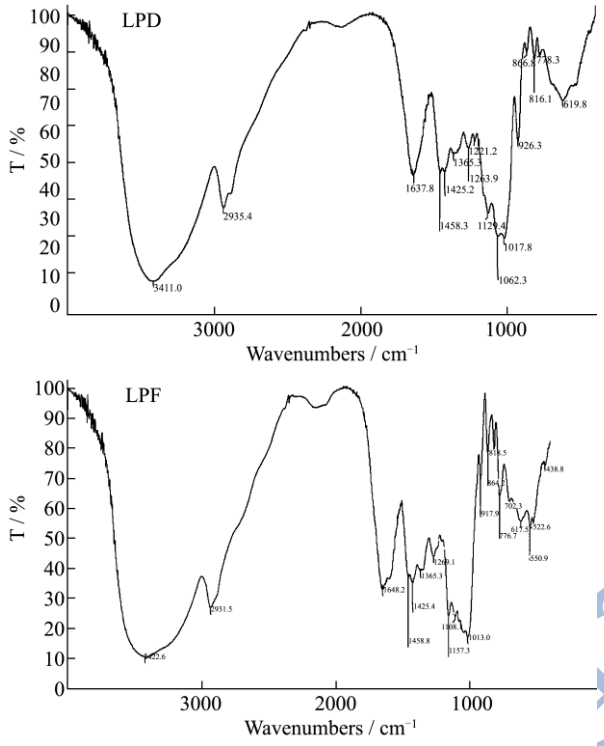

Fig.5 FT-IR spectra of longan polysaccharides LPD and LPF

2.5 Effects of longan polysaccharides on lymphocyte proliferation

The effects of LPD and LPF on the proliferation of splenic lymphocytes are shown in Fig.6. They could result in the enhancement of the normal proliferation at a dose range of 50~400 μg/mL with 11~21% and 6~18% upregulation, respectively. The SI of LPD was significantly higher than that of LPF at 50 or 100 μg/mL (P < 0.05), but the statistical difference was not found at higher doses (P > 0.05). In addition, Longan polysaccharides led to the stimulation of ConA-induced lymphocyte proliferation in the tested dose range (P < 0.05), but LPD and LPF had no statistical difference in the *SI* (P>0.05), except at a dose of 200 μg/mL. Their optimal stimulating effects on normal and ConA-induced proliferation were both 200 μg/mL. However, only 50 μg/mL of LPF could stimulate LPS-induced proliferation (P < 0.05).

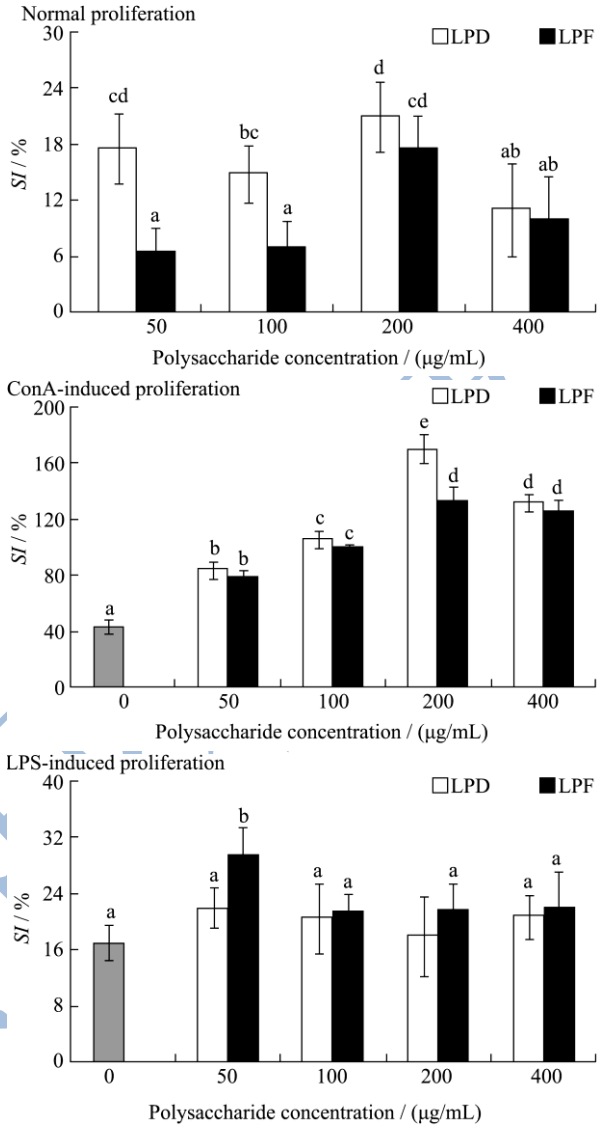

Fig.6 Effects of longan polysaccharides (LPD and LPF) on the proliferation of splenic lymphocytes

Note: The stimulation indices (*SI*) were expressed as means ± SD (n = 6). The statistically significant differences among the groups were evaluated with ANOVA followed by the S-N-K test. Different letters in the figure represented the statistical difference at P < 0.05.

2.6 Effects of longan polysaccharides on macrophage phagocytosis

In comparison with LPS, LPD and LPF showed stronger enhancement of macrophage phagocytosis (P < 0.05), as seen in Fig.7. The *PI* of LPD and LPF significantly decreased with increasing polysaccharide doses (P < 0.05). At the same dose, the *PI* of LPD was obviously higher than that of LPF (P < 0.05).

2.7 Effects of longan polysaccharides on macrophage NO production

As seen in Fig.7, LPD and LPF displayed stronger enhancement of NO production in macrophages compared to LPS ($P < 0.05$). The *NI* of LPD in the dose range of 50~400 μg/mL showed no statistical difference ($P > 0.05$). For the *NI* of LPF, there was no notable difference between 50 and 100 μg/mL doses ($P > 0.05$), but there was a significant decrease at higher doses ($P < 0.05$). At any given dose, the *NI* difference between LPD and LPF was not significant ($P > 0.05$).

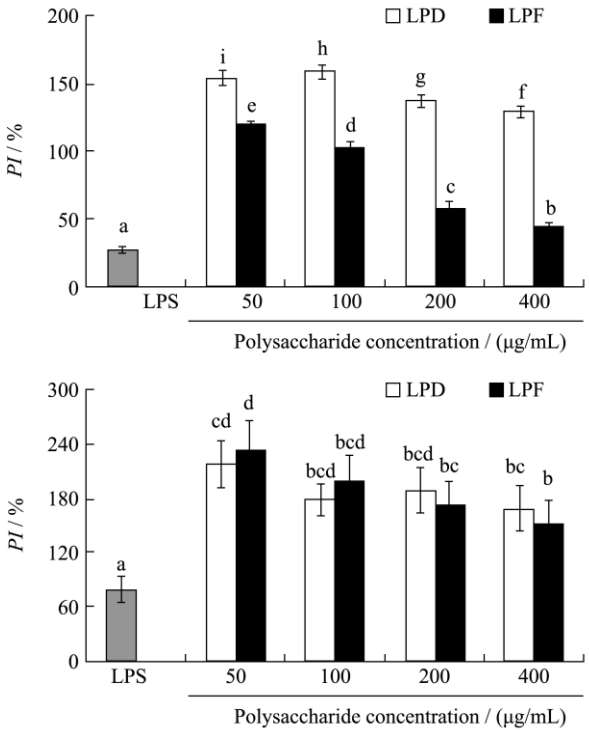

Fig.7 Effects of longan polysaccharides (LPD and LPF) on the phagocytosis and NO production of macrophages

Note: The phagocytosis indices (*PI*) were expressed as means ± SD (n = 6). Their NO production indices (*NI*) were evaluated using the Griess test and expressed as mean ± SD (n = 4). The statistically significant differences among the groups were evaluated with ANOVA followed by the S-N-K test. The different letters in the figure represented the statistical difference at $P < 0.05$.

ConA-induced lymphocyte proliferation was used to evaluate the activation of T lymphocytes, while LPS-induced proliferation was used to evaluate that of B lymphocytes [25~27]. LPD and LPF exhibited excellent stimulations on ConA-induced proliferation, but weakly stimulated LPS-induced proliferation. It was suggested that they selectively enhanced T-cell activity. The results were inconsistent with those of previous investigations on the purified polysaccharide fraction of longan pulp, which were reported to depress ConA-induced lymphocyte proliferation and promote LPS-induced lymphocyte proliferation[4]. Besides, LPD showed stronger stimulation on ConA-induced lymphocyte proliferation and macrophage phagocytosis compared to LPF. The involved mechanism was partly related to the differences in chemical composition and molecular weight. The polysaccharides of *Lycium barbarum* L. binding a large number of proteins displayed stronger activation of T cells, and their effects significantly decreased with protein digestion [28]. In contrast, *Acanthopanax koreanum* polysaccharides could selectively stimulate B cells, and their promotion of lymphocyte proliferation did not decrease with protease K induced protein digestion [29]. It was suggested that the binding proteins of LPD were beneficial for T cell activation. LPD and LPF could effectively promote the phagocytosis and NO production by macrophages. Similarly, polysaccharides or proteoglycans from *Misgurnus anguillicaudatus* [26] and *Lycium barbarum* [28] selectively activated T cells and macrophages, but did not affect B cells directly.

It was found that the effects of polysaccharides on lymphocytes were closely related to the branch of molecular chain mainly composed of D-mannopyranosyl residues, D-galactopyranosyl residues, and D-glucopyranosyl residues [4]. Arabinose, galactose, and mannose were considered to play important roles in immunostimulation [30,31], which might contribute to enhanced immunomodulatory activity of LPD. In addition, the average molecular weight of LPD was higher than that of LPF. The polysaccharide with higher molecular weight usually exhibited stronger immunostimulation, such as those from *Opuntia polyacantha* [32]. Polysaccharides with larger molecular weights might have more glyco-ligands, which could be recognized by immune cell receptors[33].

3 Conlusion

LPF and LPD isolated from fresh and dried longan pulp, and both were polysaccharide-protein complexes; but their protein-binding contents, neutral/acid polysaccharide ratios, monosaccharide compositions, molecular weight distributions, and intrinsic viscosities showed significant differences. They could significantly

stimulate ConA-induced lymphocyte proliferation and macrophage NO production, but could only weakly stimulate LPS-induced lymphocyte proliferation. The better immunomodulatory effects of LPD compared with those of LPF were potentially related to the drying process of the longan pulp. The mechanism of action of thermal drying on the longan pulp polysaccharides mainly involved in the relationship between the structure and immunomodulatory activity needs to be further explored. Further, the effects of different drying methods should also be investigated.

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