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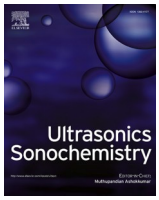

Ultrasonic/enzymatic extraction, characteristics and comparison of leechee peel polysaccharide

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ABSTRACT

In this study, the process conditions, physicochemical properties, structural composition and activity of polysaccharides isolated from leechee peel (LPP) by ultrasound-assisted extraction (UAE) with enzyme and alkali solution extraction (ASE) were compared. The results showed that the total sugar content of LPP extracted by UAE accounted for 75.65 %, which was significantly higher than that extracted by alkali solution. The optimum conditions were as follows: extraction temperature of 68.78 ℃, ultrasonic enzymolysis time of 39.68 min, pectinase dosage of 4.03 %, solid–liquid ratio of 1:30 g/mL, and ultrasonic power of 360 W. The antioxidant activities and structure of leechee peel polysaccharide (LPP) prepared under different conditions were compared. It was found that UAE-LPP was an α-type polysaccharide containing 15.83 % uronic acid. Moreover, LPP extracted by UAE showed strong activity in anti-lipid peroxidation and reducing ability. Ultrasound-assisted enzymatic method is an effective means to improve the content and activity of natural plant polysaccharides, and this method has the advantages of short time-consuming, simple process and easy operation, which can greatly improve the utilization rate of polysaccharides and lay a theoretical and scientific basis for the development and utilization of LPP.

1. Introduction

Leechee belongs to a seasonal tropical fruit, which is rich in a variety of vitamins and minerals [1]. Leechee flesh is very tasty and enjoyed by people, but it is susceptible to browning and has a shorter shelf-life [2]. People will be processed into canned meat, leechee wine or drying the fruit dried meat to save, and most of the peels as food waste disposal, is a tremendous waste of resources, and placed for a long time will produce rotting odor, if not handled well, but also to the surrounding environment to cause even greater pollution [3]. Polyphenols extracted from orange peel waste had antioxidant activity [4]. Extracts from banana peel powder could effectively alleviate anxiety symptoms [5]. Pomegranate peel contained a large number of bioactive substances such as phenols and flavonoids, which had strong antioxidant activity and antibacterial effect [6]. These peel wastes, which were often discarded, had become the source of valuable products. Recycling and effectively processing them, turning waste into treasure, not only could reduce the damage to the environment, but also the active substances in the peel were beneficial to human life and health. Therefore, this paper was also of great significance to the development and utilization of leechee peel [7]. The weight of leechee peel accounts for about 18 % of the whole fruit. Leechee peels contain flavonoids, polysaccharides, phenols and other active ingredients [8], with anti-inflammatory, anti-aging, immunomodulation, anti-cancer, memory enhancement and other functions [9]. However, the current research on leechee mainly focused on leechee pulp, seed polysaccharides, proanthocyanidins, polyphenols, and flavonoids in leechee peels [10]. In contrast, there had been few studies on peel polysaccharides. Therefore, it was of great significance to isolate polysaccharides from leechee peel and study their structural characterization and physiological activities.

At present, there were few studies on the extraction conditions, structural characterization and biological activities of leechee polysaccharide by ultrasound-assisted extraction (UAE) [11,12] and alkali solution extraction (ASE) technology [13], and the yields and conformational relationships of leechee peels extracted by UAE and ASE still need to be investigated. Therefore, this research aimed to improve the yield of leechee peel polysaccharide (LPP) and maximize the utilization of waste by ultrasonic effect, enzyme degradation and alkaline

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environment. However, these two techniques also had their shortcomings. For example, the ultrasonic intensity was too high, or the polysaccharide was hydrolyzed in a higher alkaline environment, the yield was reduced, and the biological activity was also changed [14]. Thus, it was crucial to optimize the UAE method and ASE method to obtain higher yields of LPP, and, furthermore, the impacts of different fabrication process on the structural characteristic and activity for LPP were explored and compared [15].

Response surface methodology (RSM) was a combination on statistics mix together mathematics. It had been successfully applied to ascertain the mutual interaction of multiple variables and the optimal process [16,17]. RSM was a correlation model capable of identifying correlations between multiple input variables and one and more response values, reducing the number of trials, evaluating interactions between multiple covariates, and having high computational accuracy and efficiency [18,19]. In this study, UAE manufacturing method and dilute alkaline extraction processes were optimized using Box-Behnken design (BBD), respectively. The three-factor, three-level BBD was used as the basis for the experiment, and the effects of different temperatures, different times, enzyme additions, and different alkali concentrations on the LPP yield were investigated separately.

2. Materials and methods

The mature leechee variety was selected from the Lingnan area of Guangdong Province, China. The leechee peel was dried to constant weight (brown, thin, crisp, curly) and crushed through a 70-mesh sieve as raw material. Pectinase (BR, 500 u/mg) was purchased from Shanghai yuanye Bio-Technology Co., Ltd. Papain (food grade, 100,000 u/g) was purchased from Beijing Solarbio Science & Technology Co., Ltd. Cellulase (98 %, 10,000 u/g) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Other related reagents were purchased from Chongqing Chuandong Chemical Co., Ltd. and were of analytical purity grade.

2.1. Preparation of LPP

2.1.1. Pretreatment

Taking the sieved leechee peels and soak them in anhydrous ethanol for 1 h at room temperature (raw material: anhydrous ethanol = 1:20 g/mL), Then it was refluxed at a temperature of 60 ℃ for 2 h, degreased and decolorized [20], cooled. This was followed by filtration and drying of the sludge. It was dried at 50 °C for approx. 6 h until there was no further change in the quality of the leechee husk powder, reaching constant weight, and keep them in a ziplock bag for subsequent utilization.

2.1.2. Ultrasonic-assisted enzymatic extraction of LPP

Experimental procedure for extracting LPP: A certain mass of pretreated leechee peel powder was weighed and put into a dry beaker. Then add a certain amount of enzyme and solid–liquid ratio of ultrasonic extraction of LPP, the ultrasonic frequency of 40 kHz. The mixture in the beaker is preheated in an ultrasonic machine for 5 min before ultrasound to activate the enzyme [21]. After ultrasonic treatment was completed, the polysaccharide extract was extracted and filtered. It was then vaporized to 1/5 of its previous volume using a whirling evaporator at 53 ℃ under reduced pressure [22]. After the mixture was cooled, anhydrous ethanol was added, and then the mixture was placed in a refrigerator at 5 ℃ for 12 h [23,24]. Next, the alcohol precipitation product was separated from ethanol by centrifugation, leaving only the bottom sediment.

Test procedure for protein removal: adding distilled water in small amounts several times to the bottom precipitate collected above to dissolve the alcoholic precipitate thoroughly, adding it to the separatory funnel at the ratio of extract: trichloromethane: *n*-butanol = 15:4:1 and shaking vigorously to discard the proteins in the middle layer and the organic solvent in the bottom layer [25]. The separated upper solution was then passed through centrifugation to further remove the protein and organic layers and the supernatant was collected. The extract was dialyzed in distilled water for 2 days (3500 Da dialysis bag) to remove oligosaccharides and small molecular compounds, and freeze-dried to obtain UAE-LPP.

2.1.3. Alkali solution extraction of LPP

Weighing a certain mass of defatted leechee shell powder, and finite concentration of dilute alkali solution was added to extract the mixture at a certain temperature and time [26]. The extract was allowed to cool and then centrifuged, filtered, the filtering cloth medium was collected, and the extract was adjusted to be neutral using an acid-base pH meter. Using a rotary evaporator, the volume of the mixed solution was evaporated to about 20 mL at 55 ℃ under a safe degree of vacuum, and then cooled [27]. Using graded alcohol precipitation, anhydrous ethanol was added to the concentrate ($V_{extract}$: $V_{anhydrous\ ethanol}$ = 1:3), which was stored in a refrigerator at 5 ℃ for 6 h and centrifuged, alcohol precipitate was discarded, the ethanol mixture was retained and vortexed. Anhydrous ethanol was added for the 2nd time (V extract: V anhydrous ethanol = 1:4), which was frozen at low temperatures for 12 h. The alcoholic sediment was retained by centrifugation [28]. Refer to 2.1.2 for the steps of protein removal, and finally freeze-dry in cold dryers in order to obtain LPP extracted by alkali solution (ASE-LPP).

2.2. Determination of LPP composition content

The total sugar content was determined by phenol–sulfuric acid method, which was modified according to the method of Yue et al. [29]. Different volumes of 0.1 mg/mL glucose solution (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 mL) were added, and then distilled water was added to make up 1 mL. Add 1 mL 5 % phenol solution and 5 mL concentrated sulfuric acid, and reacted for 20 min at 50 ℃. And the absorbance values of different concentrations of standard solutions were determined by UV spectroscopy at 490 nm [30,31]. The protein content in the sample was determined by Coomassie brilliant blue-G250 method, and the method was modified with reference to Grintzalis et al. [32] and other methods. Added 1 mL of different concentrations bovine serum albumin standard solution (0.0, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 mg/mL), and then added prepared 5 mL Coomassie brilliant blue solution sequentially, shaken evenly, reacted at 30 ℃ for 5 min. The reaction was cooled to ordinary-temperature and the absorbance values of standard solutions of different concentrations were measured at λ = 590 nm. The content of uronic acid in LPP samples was determined by carbazole-sulfuric acid method. The method was modified slightly according to the method of Dacheng et al. [33] and Shen et al. [34]. Weigh a certain mass of $Na_2B_4O_7{\cdot}5H_2O$ dissolved in sulfuric acid (dissolved in boiling water bath) and condense. Different volumes of galacturonic acid solution (0.0, 0.1, 0.3, 0.5, 0.6, 0.7, 0.9 mL) were added in turn and added distilled water to make up the volume of 1 ml. Subsequently, 6 mL of borax-sulfuric acid solution was added, shaken well. They was put in an environment of 90 ℃ for a period of time, followed by the addition of 0.2 mL 1.25 % carbazole solution, shaken well. Next, the mixture was continued to put in a high temperature environment of 90 °C for 10 min, and cooled to 20 ~ 25 ℃. The absorbance was measured at 520 nm. The total phenol content in the sample was determined by Folin-Ciocalteu method [35]. It was modified according to the method of Platzer et al. [36]. Different volume gradients of gallic acid standard solution were placed in 9 mL dry centrifuge tUAE. Further added the 5 mL of Folinol solution (1 mol/L, 10 %) respectively and mix well, and reacted for 5 min away from light. Finally, 4.0 mL Na_2CO_3 solution (7.5 %, w/v) was added and reacted for 40 min in the dark. The final solution color changes in a concentration gradient, from light to dark brown. The absorbance was measured at 765 nm.

2.3. Optimization of UAE of LPP

UAE was used to extract polysaccharides from leechee peels to investigate the effects of five factors, namely, the type of enzyme, the amount of enzyme, the extraction temperature, the ultrasonic enzyme digestion time, and material-fluids ratio, on polysaccharide content of leechee peels. The polysaccharide content of leechee husk extracted by UAE method was used as an index level. The factors that had a great influence on the yield of polysaccharides in the single factor experiment results were compared and analyzed. The Design-Expert 13 software was used to design the response surface experiment to further determine the optimal extraction conditions.

2.3.1. UAE of LPP single factor experiment

(1) Effect of material-liquid ratio on UAE of leechee husk polysaccharide content. Five 100 mL dry beakers were selected, 1 g of pretreated leechee peel material and 1 % enzyme dosage were added, respectively. In the case of fixed ultrasonic power and other conditions unchanged, different material-liquid ratios (Table 1) were added to the beaker to investigate influence of different material-fluidity ratios on the content of leechee husk polysaccharides extracted by ultrasonic-assisted enzyme. The extraction operation was referred to 2.1.2, and the optimal material-liquid ratio was selected according to the UAE-LPP content as a fixed condition for the next one-way experiment.

(2) Influence of sonication period of time on the content of LPP extracted by UAE. Weigh 1 g of pretreated leechee husk powder separately, add it to six numbered beakers, add equal volume of 30 mL distilled water and 1 % enzyme dosage according to the material-liquid ratio m (raw material): V (distilled water) = 1: 30 g/mL, and mix it uniformly. Six sets of parallel experiments were conducted to investigate the effect of time-consuming on the content of LPP by UAE while fixing other extraction environments in a consistent manner. The extraction operation was referred from 2.1.2, the most favorable consumption time was selected according to the UAE-LPP content as the fixation condition for the next one-way experiment.

(3) Effect of enzyme dosage on UAE of LPP content. Weighing 1 g of defatted treated leechee husk powder and varying the enzyme dosage under fixed conditions of feed to liquid ratio, sonication time, temperature, and sonication power, as shown in Table 1. Five sets of parallel experiments were conducted to investigate the effect of enzyme dosage on the content of leechee husk polysaccharides in UAE. The extraction operation was referred to 2.1.2, and the optimal required enzyme dosage was selected based on the UAE-LPP content as the fixation condition for the next unifactorial experiment.

(4) Effect of extraction temperature on polysaccharide content of leechee husk extracted by UAE. UAE-LPP polysaccharides were extracted from 1 g of defatted leechee husk powder by varying the factor of temperature under the fixed conditions of material-liquid ratio, ultrasonic time, ultrasonic power, and enzyme dosage. Since the activity of the enzyme is affected by temperature and higher temperatures may lead to inactivation of the enzyme, a temperature gradient change as shown in Table 2 was set to extract UAE-LPP within the temperature range of enzyme activity. Do four sets of parallel experiments. To explore and discuss how the temperature required in the process of UAE fabrication for LPP affected the content of LPP. The extraction operation was referred to 2.1.2, and the optimal extraction temperature was selected according to the content of UAE-LPP.

Table 1
ASE of LPP one way experiment.

Research on single factor						
Ratio of solid to liquid g/mL	1:15	1:20	1:25	1:30	1:35	1:40
NaOH solution concentration / %	1	2	3	4	5	6
Time / min	30	45	60	75	90	105
Temp / ℃	20	30	40	50	60	70

Table 2
UAE of leechee husk polysaccharide one way experiment.

Single-factor inquiry						
Temperature / ℃	45	55	65	75		
Ratio of feed to liquid / g/mL	1:15	1:20	1:25	1:30	1:35	
Amount of enzyme / %	2	3	4	5	6	
Time /min	30	40	50	60	70	80

2.3.2. UAE of LPP response surface experiments

In accordance with the Box-Behnken principle, the required warmth, enzymolysis chronos and enzyme dosage were arranged as the changing conditions, and carry out the experimental design shown in Table 3.

2.4. Optimization study of ASE with LPP

The polysaccharide in leechee peel was extracted by alkali extraction and ethanol precipitation method. The effects of NaOH solution concentration, extraction time, temperature and solid–liquid ratio on the yield of polysaccharides from leechee peel were investigated. Comparison of ASE-LPP content as determined through the phenol–sulfuric acid means. Analyzing and selecting these zetetic factors that had greater impact on the polysaccharide yield in the results of single-factor experiments, the Design-Expert 13 software was used to design the response surface experiment to further determine the optimal extraction conditions.

2.4.1. Single factor experiment of LPP by ASE

(1) Effect of solid–liquid ratio on the yield of polysaccharide extracted from leechee peel by alkali solution. Taking 1 g of defatted leechee husk powder, different material-liquid ratios were explored at a certain temperature T = 60 ℃, time t = 60 min, and 3 % NaOH solution, as shown in Table 1. To assay the effect of alkaline solution on the extraction output capacity of leechee husk polysaccharides. The extraction procedure was referred to 2.1.3, and the classic material-liquid ratio was selected according to the ASE-LPP content as the fixation condition for the next one-way experiment.

(2) Effect of concentration of sodium hydroxide solution on yield of polysaccharide from leechee pericarp. Weighing 1 g of dry pretreated leechee husk powder, the extraction temperature was fixed at 60 ℃, the fixation time was 60 min, and the material-liquid ratio was 1:25 g/mL, to investigate the effect of different NaOH solution concentrations on the yield of leechee husk polysaccharides extracted from the alkaline solution. The extraction procedure was referred to 2.1.3, and the optimal base solution concentration was selected based on the ASE-LPP content.

Table 3
Box-Behnken test factors and levels for UAE-LPP.

Levels	Considerations		
	A Temperature / ℃	B Ultrasonication time / min	C Amount of enzyme / %
−1	55	30	3
0	65	40	4
1	75	50	5

(3) Effect of extraction time on the yield of LPP extracted from dilute solution of NaOH. Weighing 1 g of defatted and dried leechee husk powder, adding 3 % NaOH solution under the conditions of ratio of raw materials to water of 1:25 g/mL and extraction temperature of 60 ℃, the extraction time was varied to explore how the time affects the content of alkaline extracted leechee husk polysaccharides. The classic extraction time was selected according to the ASE-LPP content.
(4) To investigate the effect of temperature on the yield of alkali extracted LPP. The extraction temperature was varied according to a certain gradient (Table 1). Under the fixed factors of material-liquid ratio, temperature, and NaOH solution concentration (1:25 g/mL, 60 ℃, 3 % NaOH solution), and compared how different extraction times affected the alkaline extraction of leechee husk polysaccharide content. The optimal extraction temperature was selected based on the ASE-LPP content.

2.4.2. Response surface experiment of extracting LPP by ASE

Based on a single factor test, alkali solution concentration, time consumed, and temperature required were arranged as three most influential moments, ASE-LPP yield R_1 was the response value. Using design principles, the extraction technology was optimized by the effect surface method. The experimental design was carried out with 3 factors and 3 levels. In Table 4 the result was summarized.

2.5. Structure characterization

2.5.1. FT-IR analysis

The crude polysaccharide sample powder obtained by the two extraction methods was accurately weighed 3 mg, and the two crude polysaccharides of UAE-LPP and ASE-LPP were analyzed by FT-IR using potassium bromide (KBr) compression method. Scanning was in the range of 4000–500 cm^{-1}.

2.5.2. NMR analysis

NMR was one of the most useful tools for analyzing polysaccharide structure, could elucidate polysaccharide structural information, including the composition of monosaccharides, heterocarbon configurations (α, β), and the mode of glycosidic bonding [37]. The technology had also developed rapidly in recent years. The pretreated leechee husk powder was weighed separately and extracted under the optimum process conditions of two different extraction methods. The precipitate obtained after alcohol precipitation was dissolved, deproteinized, dialyzed and other experimental operations, and then concentrated under reduced pressure until the volume of the concentrate was about 1 mL, obtaining two types of crude polysaccharide concentrates, UAE-LPP and ASE-LPP. After centrifugation, 0.36 mL of the supernatant of the sample polysaccharide concentrate was taken in a NMR tUAE, respectively, and 0.03 mL of D_2O (99.99 %) was added, mixed, and analyzed by carbon spectroscopy C13CPD using 300 MHz NMR.

2.6. Study on antioxidant activity of LPP

Explore whether polysaccharides had some antioxidant activity, antioxidants gave electrons through their own reduction for the purpose of scavenging free radicals, the greater the ability to scavenge free radicals. In other words, the greater the reducing power, the greater the antioxidant capacity of glycan [38]. In this experiment, three different antioxidant competences, hydroxyl radical scavenging capacity, reducing capacity and anti-lipid peroxidation capacity of leechee polysaccharide were analyzed by measuring the absorbance values at specific wavelengths using UV assay.

Table 4
Box-Behnken test factors and levels of ASE-LPP.

Levels	Test factors		
	A Alkaline concentration / %	B Extraction time / min	C Temperature / ℃
−1	2	75	50
0	3	90	60
1	4	105	70

2.6.1. Determination of hydroxyl radical (·OH) scavenging ability of LPP

Free radicals did not have the ability to distinguish, when the body was synthesized in large quantities, the first attack was the cell membrane. When the free radicals took away the electrons, the cell membrane would lose its elasticity, lose all the functions, triggering cardiovascular, Alzheimer's disease, liver disease, eye disease, cancer, inflammation, skin aging, and many other diseases [39]. More than two hundred diseases had been linked to the production of free radicals. It was of great importance to explore this scavenging of free radicals by antioxidants.

The Fenton reaction uses H_2O_2 as the oxidizing agent and produces ·OH catalyzed by Fe^{2+}. The numerical values were tested at 510 nm after the action about LPP solution with the generated hydroxyl radicals, and this clearance for the LPP on ·OH could be indirectly judged by the change of the absorbance value. According to the experimental method of Zhou et al. [40], some modifications were made. 1 mL of different mass concentrations of polysaccharide solution (0, 0.3, 0.9, 1.5, 2.1, 2.4, 2.7, 3.0 mg/mL) were added with 1 mL of $FeSO_4$ solution, 1 mL of salicylic acid–ethanol solution and 1 mL of H_2O_2 solution, respectively, and reacted at 37 °C for 30 min. Ascorbic acid (Vc) was used as positive control. Distilled water as blank experiment. The ·OH scavenging efficiency of LPP was calculated by the following method:

$$\text{OH scavenging rate } (\%) = [1 - (A_0 - A_s)/A_0] \times 100\%$$

In the formula, the experimental group was noted as A_s, with distilled water as the lacuna matched group (A_0).

2.6.2. Antilipid peroxidizing capacity activity of LPP

The thiobarbituric acid method was based on the formation of oxidizing radicals from unsaturated fatty acids through a free radical reaction, which oxidized to produce epoxy compounds. The epoxy compound decomposes to form malondialdehyde (MDA), which reacted with TBA to form a pink TBA dye compound that had a maximum absorption value at 535 nm. Therefore, the amount of scavenging of oxidized radicals by LPP, i.e., the measured absorbance value, was a measure of the extent to which the oxidation reaction of the free radical chain had proceeded. Referring to the experimental method of Wang et al. [41], a slight modification was made. Soybean lecithin solution (1 mL) and $FeSO_4$ solution (0.4 mL, 10 mmol/L) were added to 1 mL polysaccharide solution with different mass concentrations (0, 0.3, 0.9, 1.5, 2.1, 2.3, 3.0 mg/mL), and the reaction was carried out at 37 °C for 50 min. Then Cl_3CCOOH (20 %, w/v) and thiobarbituric acid solution (0.8 %, w/v) were added respectively, and the reaction was carried out at 95 °C for 20 min. After cooling and centrifugation, the supernatant was taken and the absorbance was measured at 535 nm. The ascorbic acid was used as the normal control [42]. The anti-lipid peroxidation performance of LPP was calculated according to the following method:

$$\text{Anti - lipid peroxidation ability } (\%) = [(A_0 - A_s)/A_0] \times 100\%$$

A_0: Distilled water and a certain volume of the added reagent were used as the blank control group; A_s as the experimental group.

2.6.3. The reducing power of LPP

Polysaccharide had a certain antioxidant activity, which could reduce Fe^{3+} in potassium ferricyanide solution to Fe^{2+} and produced potassium ferrocyanide. The buffer was added to ensure that the reaction could proceed at a certain pH. The reaction was carried out for a period of time to ensure that the polysaccharide solution reacted completely and the remaining potassium ferricyanide from the reaction

reacted with trichloroacetic acid to form a precipitate, which was removed by centrifugation to exclude any effect on the absorbance values. Potassium ferricyanide further reacted with ferric chloride to form Prussian blue ($Fe_4[Fe(CN)_6]_3$) with maximum absorbance at 700 nm. Hence, the level at 700 nm could indirectly reflect the reducing ability of polysaccharides, and the higher the absorbance, the stronger the reducing ability [43]. Referring to the experimental method of Song et al. [44], a slight modification was made. 2.5 mL phosphate buffer (PBS, pH = 6.6) and 2.5 mL $K_3Fe(CN)_6$ solution were added to 1 mL polysaccharide solution with different mass concentrations (0, 0.3, 0.9, 1.5, 2.1, 2.4, 2.7, 3.0 mg/mL). Distilled water was diluted to 10 mL, 50 °C and reacted for 30 min. After cooling, 2.5 mL Cl_3CCOOH (10 %, w/v) was added to terminate the reaction. Centrifugation, taking 2 mL supernatant, adding 2 mL H_2O and 0.4 mL $FeCl_3$ solution (1 %, w/v), reaction 15 min, 700 nm absorbance. The ascorbic acid was used as control. The formula was as follows:

$$\text{Reducing capability } (\%) = \left[1 - (A_s - A_0)/A_g\right] \times 100\%$$

A_s was the experimental group; A_g with distilled water as the control group; A_0 was a polysaccharide sample solution with buffer as a blank group.

3. Results and discussion

3.1. Process optimization study of LPP with ASE method

3.1.1. Effect of various factors on the extraction of LPP by ASE

Four factors, namely, ratio of raw material to liquid, alkali concentration, the required temperature and time, were selected for the experiment, and one of them was changed while the others were fixed to explore the polysaccharide content of ASE-LPP extracted by alkali method, respectively [42]. And the one-factor conditions with different gradients were taken as the horizontal coordinate, and the rate of polysaccharides yielded from ASE-LPP was taken as the vertical coordinate, as shown in Fig. 1.

The relationship between the variation of liquid to feed ratio as well as time on the sugar content for LPP was presented in Fig. 1(1) and Fig. 1 (3). The total sugar content showed a trend of increasing and then decreasing with the increase of liquid ratio as well as time, and the maximum sugar content detected was 59.56 % and 62.11 %, respectively. The reason for this phenomenon might be due to a certain amount of leechee peel raw materials in the polysaccharide content was a certain amount, and then extend the extraction time and increase the ratio of raw material to liquid, resulting in other soluble impurities were also dissolved in it, and the polysaccharide content gradually reduced [11]. Fig. 1(2) showed the effect of alkali concentration on polysaccharide content. It could be seen that the NaOH concentration increased and this extracted polysaccharide content increased and the maximum LPP sugar content was obtained when 3 % NaOH solution was added. Most of the polysaccharides were acidic polysaccharides, which could be better dissolved in alkali solution, so that the polysaccharide yield was increased, but when the concentration of NaOH added was greater than 3 %, the content of polysaccharides decreases, probably because the high concentration of alkali destroys the structure of polysaccharides. Therefore, considering all the factors, the concentration of 3 % NaOH solution was selected as the optimal single factor condition. Fig. 1(4)

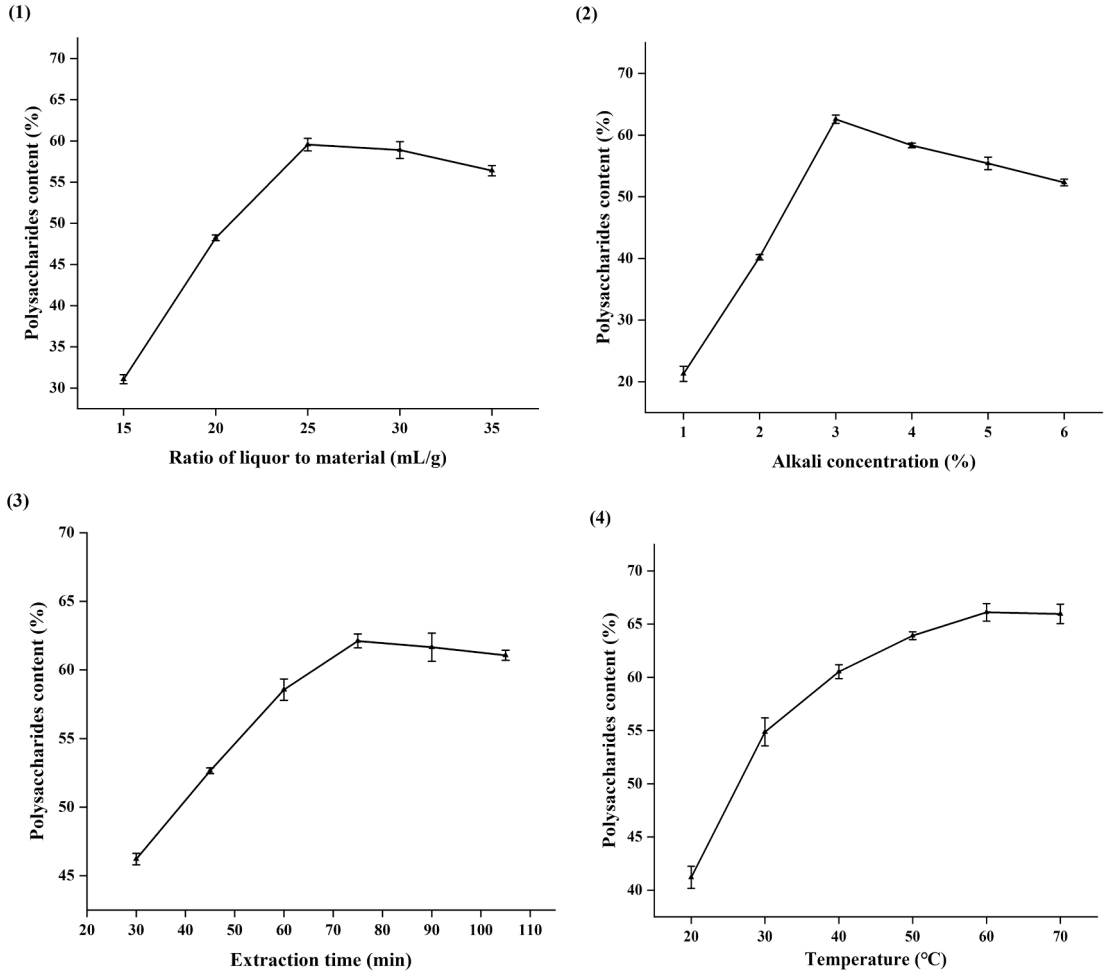

Fig. 1. Effect of various conditions on LPP content of ASE.

showed the effect of temperature on LPP sugar content. Increasing the temperature was helpful to improve the dissolution rate of LPP, and when temperature exceeded 60 ℃, the change in polysaccharide content was not obvious. Therefore, 60 ℃ was selected as the optimal extraction condition in order not to cause excessive waste of resources and energy. In addition, excessive temperature might lead to the degradation and loss of polysaccharides [14]. In summary, the analysis and results showed that the better one-factor conditions were selected as liquid-feed proportion of 25:1 mL/g, NaOH concentration of 3 %, extraction time of 75 min, temperature of 60 ℃.

3.1.2. Response surface optimization experiment planning and results

A single factor test was conducted with three factors, the alkali concentration was set to A, the consumption time was set to B and C was set to temperature, three factors as independent variables, and total sugar content as response value (R_1) [19]. Box −Benken was deployed to investigate the total sugar content of leechee husk under different extraction conditions, a total of 17 sets of exploratory experiments were conducted and the results were revealed in Table 5.

3.1.3. Regression equation fitting and analysis of variance

The experimental data in Table 5 were fitted by multivariate recurrence and a mathematical model was developed to obtain a quadratic multiple regression equation: $R_1 = -251.41175 + 5.91825*C + 4.11177*t + 4.18828*T + 0.0905*C*t + 0.021*C*T - 0.000183*t*T - 2.4095*C^2 - 0.024342*t^2 - 0.035395*T^2$ (Where C denoted the concentration of dilute alkali solution, t denoted time, and T denoted temperature). The coefficients of quadratic terms in this regression equation of the fitted quadratic model were all negative, indicating that the parabola had a downward opening and there was a point of extreme value of the response. The correlation coefficient of this equation, $R^2 = 0.9965$, demonstrated a significant linear relationship. The calibration coefficient of determination Adjusted $R^2 = 0.9925$ indicated that the experimental resulted had a good fit and reliable data. It could be used to analyze and predict experimental results. The variance analysis of the regression model was shown in Table 6. The regression model P less than 0.0001, the F numerical value of the model was 235.30, indicating that the construction of the model was significant. Under these circumstances, A, AB, A^2, B^2, C^2 were important highly significant model terms. The misfit term of the model, $P = 1.0000$, was greater than 0.05, indicating that the misfit term was not significant, suggesting that the optimized model had a high probability of matching the test. In addition, the F numerical value of each item in the regression model indicated the strength of the influence of ASE-LPP polysaccharide content by the factors, and the larger the F numerical value, the stronger the influence [16]. Among them, the alkali concentration had the greatest effect on the polysaccharide content of ASE-LPP, so the degree of influence of a single factor on the content of ASE-LPP was alkali concentration > time > temperature.

Table 5
Response surface experimental design and results.

Serial number	A: alkali concentration /%	B:Time/ min	C:Temperature /℃	R_1: Total sugar content /%
1	4	75	60	60.4
2	2	90	70	61.49
3	2	90	50	62.16
4	4	105	60	62.84
5	3	90	60	68.88
6	3	90	60	68.53
7	3	105	50	59.64
8	3	90	60	69.12
9	3	90	60	67.95
10	3	105	70	59.33
11	4	90	50	63.48
12	3	75	50	59.87
13	3	90	60	68.74
14	2	75	60	61.39
15	3	75	70	59.67
16	2	105	60	58.4
17	4	90	70	63.65

3.1.4. Analysis of the interaction of various factors in the model

The interaction term of NaOH concentration and extraction time in the regression equation was used as the response surface in Fig. 2(A-1) and contour line (A-2). Response surface plots (B-1) and contours (B-2) were made for the NaOH concentration versus extraction temperature interaction term, and response surface plots (C-1) and contours (C-2) were made for the extraction time versus temperature interaction term. To analyze how NaOH concentration, time, extraction temperature and interaction between factors affect the effect of ASE-LPP total sugar content. Each factor had an extreme value in the selected range, the steeper the slope of the surface of the 3D plot of response surface curve, elliptical shape of the height-contour line, and the greater the slope of the curve, the more significant the interaction effect of the two factors on each other, and vice versa, the smaller the effect on the total sugar content of ASE-LPP. The steep slope of the NaOH concentration vs. extraction time surface in Fig. 2(A-1) and the dense elliptical shape of the contour lines in Fig. 2(A-2) indicated a significant ($P < 0.05$) interaction between NaOH concentration and extraction time. The steeper the slope of the response surface, the elliptical shape of the contour map, and the more significant the interaction [12]. The slopes of the curves for NaOH concentration vs. extraction temperature and extraction time vs. temperature were smaller, which indicated that the interaction was not significant ($P > 0.05$), which was in keeping with the findings for regression simulation test (Table 7).

3.1.5. Optimization and verification of process conditions

It was found that the model constructed with the response surface methodology had better regression results, indicating that method could predict the total sugar content of ASE-LPP well. As per to the results of the response surface, the optimal process for extracting polysaccharides from leechee peel by alkali solution was NaOH concentration of 3.18 %, spending 90.15 min, and ambient temperature was 59.87 ℃. In this condition, three parallel experiments were conducted. The average content of total sugar in ASE-LPP was 66.23 %, which was consistent with the theoretical prediction value of 68.72 % (relative error of 2.49 %). Natural ASE-LPP was extracted from leechee peel according to the optimal process conditions, and its structure and antioxidant competence were analyzed [17].

3.2. Optimization of UAE

3.2.1. The effects of various factors on the extraction of LPP by UAE

The polysaccharides contained in leechee peels were extracted by UAE approach, and Fig. 3 illustrated the impact of five factors, namely, the type of enzyme, the amount of enzyme, the extraction temperature, the ultrasound enzyme digestion time, and the liquid-to-feed ratio, on the LPP content, respectively. The content of UAE-LPP was measured by phenol–sulphuric acid as an indicator, and the optimal single factor was determined by comparison as the fixation qualification for the next single factor experiment.

In this paper, three different types of enzyme, papain, pectinase, and cellulase, were used to extract leechee husk crude polysaccharides, respectively, keeping the rest of the conditions unchanged, and the changes in the sugar content were shown in Fig. 3(e). It could be seen that the UAE-LPP sugar content obtained from the extraction with cellulase was relatively low. While the highest sugar content of 75.37 % was extracted by ultrasound-papain followed by the polysaccharide content obtained by adding some amount of pectinase extracted accounted for 68.22 % of the crude polysaccharides of UAE-LPP. Although the content of polysaccharide extracted by papain was higher, it was found during the experiment that after a period of time of alcohol precipitation of the concentrated solution, two kinds of alcohol

Table 6
Variance analysis of regression model.

Source	Sum of Squares	df	Mean Square	F-value	P-value	significant
Model	237.88	9	26.43	235.30	<0.0001	★★
A- alkali density	6.00	1	6.00	53.44	0.0002	★★
B-time	0.1568	1	0.1568	1.40	0.2760	
C-temperature	0.1275	1	0.1275	1.14	0.3220	
AB	7.37	1	7.37	65.62	<0.0001	★★
AC	0.1764	1	0.1764	1.57	0.2504	
BC	0.0030	1	0.0030	0.0269	0.8743	
A^2	24.45	1	24.45	217.62	<0.0001	★★
B^2	126.31	1	126.31	1124.44	<0.0001	★★
C^2	52.75	1	52.75	469.61	<0.0001	★★
Residual	0.7863	7	0.1123			
Misfit term	0.0002	3	0.0001	0.0003	1.0000	–-
Pure Error	0.7861	4	0.1965			
Cor Total	238.67	16				

Annotation: –- represents no significance; ★★ indicates $P < 0.001$, meaning highly significant.

precipitates with different densities and states appeared in the solution, which were located in the upper and lower layers in solution, respectively. The large amount of alcohol precipitates in the upper layer was similar to jelly, which may be a certain pectin. After abandoning it, the total sugar content of the freeze-dried product was measured. It was found that the sugar content was significantly reduced, which was about 62.55 %. Therefore, pectinase was finally selected as an auxiliary enzyme reagent for extracting crude polysaccharide from leechee peel [19].

Fig. 3(a)-(d) demonstrate the effects about Liquid volume ratio of raw material quality, sonication time, enzyme dosage, and extraction temperature on the extracted leechee husk crude polysaccharide sugar content, respectively. In Fig. 3(a), in the range of 15:1 to 30:1 mL/g of liquid/feed ratio, the sugar content increased as the liquid/feed ratio kept increasing so that more polysaccharides were dissolved in the water. The UAE-LPP content decreased when the volume of water added exceeded 30 mL. It might be because the increase in the volume of water leads to more other soluble substances being extracted and the solubility of polysaccharides is reduced, so the proportion was selected as 1:30 g/mL [22]. In Fig. 3(b), the sugar content ascended with the increase for ultrasonic enzymolysis time, but if the time exceeded 40 min, the sugar content decreased and showed a downward trend. It might be because that the ultrasonic time was too long, which would destroy the structure of the large molecular weight polysaccharide and change it, but reduced the extraction rate of LPP, so the better ultrasonic-enzymatic hydrolysis time was set to 40 min [23]. Fig. 3(c) was the effect of the amount of enzyme on the extraction rate of LPP. With the addition of pectinase, the content of LPP increased. It might be because of the specificity of the enzyme, the pectinase decomposes the pectin in the leechee peel, so that high molecular weight LPP was dissolved in the aqueous solution, which increased the extraction rate. Therefore, the better enzyme dosage was 4 % [19]. As shown in Fig. 3(d), the effect of temperature on the sugar content of LPP in the range of 45 ~ 75 ℃ showed a trend of increasing first and then decreasing. The activity of pectinase was affected by temperature, if the extraction temperature exceeds the activity temperature of pectinase, the enzyme would lose its competence and affect the extraction rate of LPP, so temperature was changed in the suitable range of pectinase to investigate the effect of temperature on the extraction rate of LPP. The data expressed that the LPP sugar content was greatly affected by the temperature, which would not only affect the enzyme activity, but also the polysaccharide content, and the extracted LPP sugar content was the highest when the temperature was 65 ℃, so 65 ℃ was set as the better temperature for extracting LPP crude polysaccharide. In summary, based on the data of each group of single-factor experiments, and synthesizing the extraction efficiency and yield of leechee husk crude polysaccharides, the better single-factor conditions were as follows: pectinase was used as an auxiliary enzyme for the extraction of UAE-LPP crude polysaccharides, and the material-liquid ratio was selected to be 1:30 g/mL, the ultrasonic-enzymolysis time to be 40 min, the dosage of pectinase at 4 %, and the extraction temperature to be 65 ℃.

3.2.2. Response surface optimization test design and results

Based on the single factor experiment, according to the Box-Behnken center combination, the experimental design of 3 factors and 3 levels was carried out [18]. The three factors of extraction temperature (A), ultrasonic time (B) and enzyme dosage (C) were set as independent variables, and the total sugar content was the response value (R_1). A total of 17 groups of inquiry experiments were carried out, and these data were presentated in Table 8.

3.2.3. Regression equation fitting and variance analysis

The experimental data in Table 8 were fitted by multiple regression, and mathematical model was established. The regression equation was obtained: $R_1 = -502.61237 + 15.06690*T + 3.2199*t + 19.419*E + 0.0039*T*t + 0.0245*T*E + 0.056*t*E - 0.120205*T\hat{}2 - 0.046805*t\hat{}2 - 2.8955*E\hat{}2$ (Where T stood for temperature, E stood for the amount of enzyme added, and t stood for time consumed), $R^2 = 0.9965$, Adjusted $R^2 = 0.9919$. It was proved that the response surface effect had a high degree of fitting with the experiment, and this regression model variance analysis was presented in Table 7 [11]. The $P < 0.0001$ of the model indicated that the constructed model was significant. Misfit terms of the model was $P = 0.2947 > 0.05$, indicating that it was not noticeable, which was beneficial to the model. In addition, the F-value of temperature was 170.56, the F-value of time was 4.03, and the F-value of enzyme dosage was 0.1397. Comparing the F-values of the factors, it could be seen that the temperature had the most prominent effect on the UAE-LPP sugar content. The results showed that the extent of each single factor on UAE-LPP sugar content was temperature, time, and pectinase dosage in descending order.

3.2.4. Reciprocity analysis of factors in the pattern

The steeper the slope of the response surface, the elliptical shape of the contour map, and the more significant the interaction [12]. Fig. 4 reflected the effect of the interaction between temperature (A), sonication time (B), and pectinase dosage (C) on the UAE-LPP content. Among them, the response surfaces of extraction temperature and ultrasound-enzymatic digestion time (Fig. 4-1-1) had larger slopes and elliptical contours (Fig. 4-1-2), indicating that AB and AC interacted more significantly. The interaction of extraction temperature and pectinase dosage (Fig. 4-2-1) did not affect UAE-LPP content as much as the interaction of extraction temperature and sonication-enzymatic digestion time. In contrast, the slopes of the response surfaces for sonication time and pectinase addition tended to flatten out (Fig. 4-3-1) and the contour curves were rounded (Fig. 4-3-2), indicating that the BC interaction was not significant.

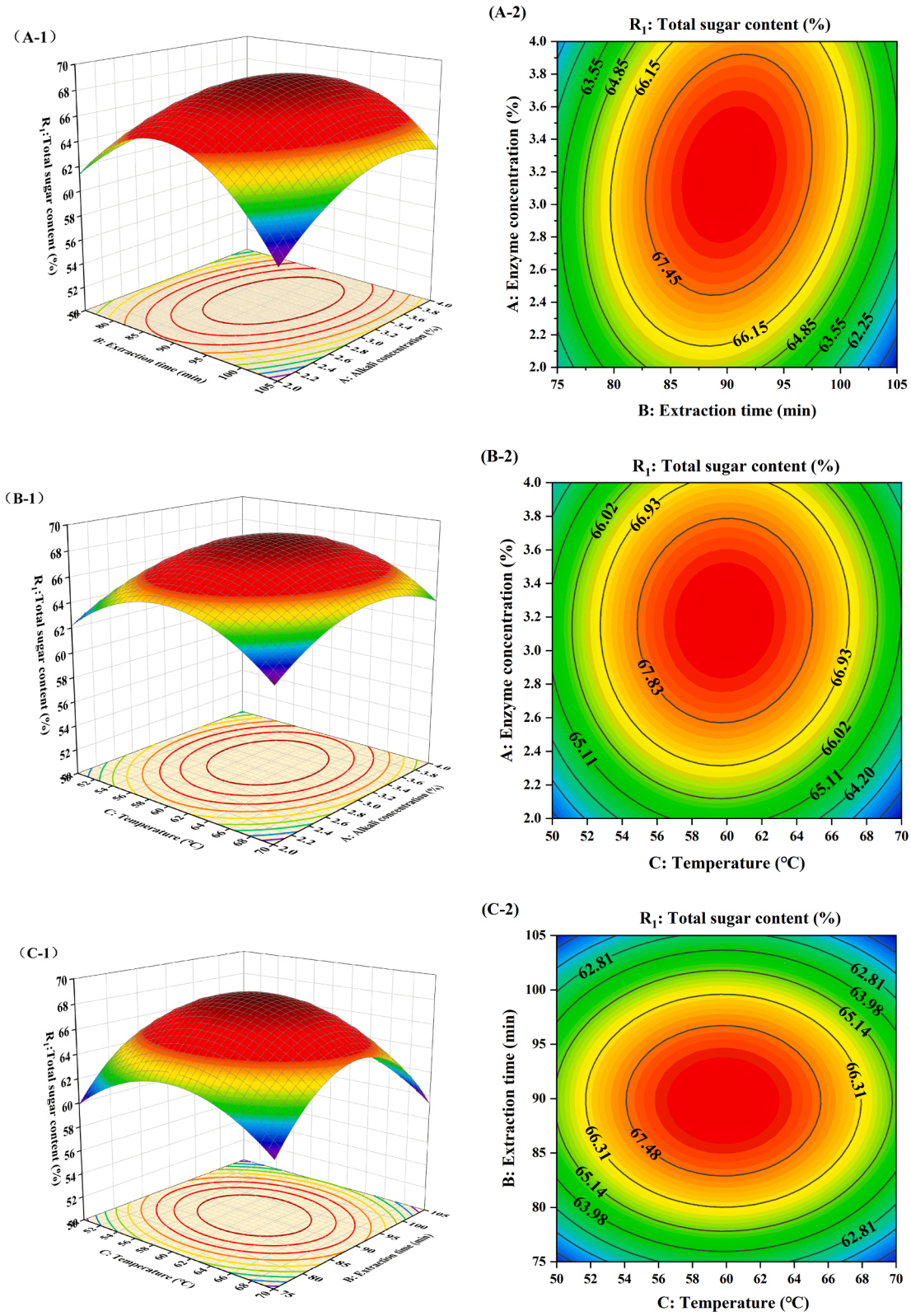

Fig.2. 3D and contour maps of the interaction of various factors in the ASE-LPP model.

3.2.5. The process conditions are optimized and validated

Through the RSM to make superior the experimental design, the optimum preparation conditions for extracting UAE-LPP polysaccharides were obtained: the extraction temperature was 68.78 ℃, UAH time was 39.68 min, and the pectinase dosage was 4.03 %. The optimum UAE-LPP polysaccharide content could reach 76.73 %. Under these conditions, the content of UAE-LPP polysaccharide was 75.65 %, and the relative error was 1.08 % compared with the theoretical value. The trial values were in preferably agreement with the frame of reference numerical values, which further verified the reliability of the regression equations and indicated that the fitting results could truly reflect these influential factors on the polysaccharide content of UAE-

Table 7
Regression model analysis of variance.

Source	Sum of Squares	df	Mean Square	F-value	p-value	significant
Model	868.21	9	96.47	220.00	< 0.0001	●●
A-Temperature	74.79	1	74.79	170.56	< 0.0001	●●
B-Time	1.77	1	1.77	4.03	0.0847	
C- Enzyme usage	0.0613	1	0.0613	0.1397	0.7197	
AB	0.6084	1	0.6084	1.39	0.2773	
AC	0.2401	1	0.2401	0.5476	0.4834	
BC	1.25	1	1.25	2.86	0.1346	
A^2	608.39	1	608.39	1387.47	< 0.0001	●●
B^2	92.24	1	92.24	210.36	< 0.0001	●●
C^2	35.30	1	35.30	80.51	< 0.0001	●●
Residual	3.07	7	0.4385			
Misfit term	1.74	3	0.5810	1.75	0.2947	—-
Pure Error	1.33	4	0.3316			
Cor Total	871.28	16				

Annotation: —- represents no marked; ●● indicates P < 0.001, meaning highly marked.

LPP, and had practical application value. The new and natural UAE-LPP was extracted from leechee husk according to the optimal process conditions and analyzed for its structure and antioxidant activity.

3.3. Effects of different extraction methods on LPP

Some studies had used hot water method to extract LPP, with polysaccharide yield as the index, to extract LPP to the maximum extent. This method was simple and easy to operate, but the gaining rate of LPP was low, and the extraction process need to be improved. Hu et al. [45] extracted and purified polysaccharides from leechee by three hot water extraction processes, and the monosaccharide components were L-rhamnose, L-arabinose and D-galactose in the ratio of 1.06: 6.39: 4.21. Gao et al. [46] used ultra-high pressure-assisted extraction of leechee polysaccharide. The experiment proved that this method could improve the yield of polysaccharide. The polysaccharide was mainly composed of rhamnose, caramel, mannose and galactose, and the molar fractions were 7.89, 46.45, 9.71 and 19.63, respectively. At the same time, it had strong reducing ability and DPPH free radical scavenging activity. Hsieh et al. [47] used porcine pancreatic amylase to remove starch and extract polysaccharide from leechee primary cell wall, and studied the structure. The results showed that leechee primary cell wall mainly contained pectin and xyloglucan, and a small amount of pure xylan. The ultrasonic extraction method could greatly improve the gaining yield of glycan, but the required temperature was higher and the time was longer. In this study, ultrasound-pectinase extraction and dilute alkaline solution extraction methods were adopted to extract leechee husk polysaccharides to obtain two polysaccharides: UAE-LPP and ASE-LPP. The optimal extraction process conditions were investigated using response surface optimization, respectively, all of which maximized the LPP crude polysaccharides containing the highest total sugar content. They were 75.65 % in UAE-LPP and 66.23 % in ASE-LPP as determined, separately. These results showed that the ultrasound-pectinase extraction method was able to extract more LPP, resulting in an increase in polysaccharide content, which was superior to the dilute alkaline solution extraction method, and was characterized by shorter time-consumption, lower extraction temperature, simplicity and high efficiency. The test proved that various methods had a certain impact on the LPP content. Therefore, it was of great value to explore different methods of LPP extraction to maximize the reuse of leechee by-product resources and avoid the waste of resources and energy.

3.4. Determination of LPP crude polysaccharide content

3.4.1. Determination of physical and chemical properties

Fig. 5(a) was the linear regression equation of glucose standard curve, $y = 0.00909x + 0.04344$, $R^2 = 0.9995$. Fig. 5(b) was the linear regression equation of bovine serum albumin standard curve, $y = 0.00403x + 0.8296$, $R^2 = 0.9991$. Fig. 5(c) was the linear regression equation of galacturonic acid standard curve, $y = 0.00446x + 0.18665$, $R^2 = 0.9990$. Fig. 5(d) was the linear regression equation of gallic acid standard curve, $y = 0.01196x + 0.02947$, $R^2 = 0.9976$.

3.4.2. Analysis of basic components of UAE-LPP and ASE-LPP crude polysaccharides

The sample polysaccharide powder UAE-LPP and ASE-LPP were accurately weighed, and 0.1 mg/mL polysaccharide solution was prepared respectively. The absorbance of 1.0 mL was measured at different wavelengths according to the experimental steps of 2.2. As per to the standard equations of the four different substance contents of the above 3.3, the basic component contents of UAE-LPP and ASE-LPP crude polysaccharides extracted under the optimization process were calculated respectively. The calculation results were shown in Fig. 6. UAE-LPP and ASE-LPP crude polysaccharides prepared by different extraction methods contained polysaccharides, proteins, uronic acids, phenolic compounds and other substances, among which polysaccharides accounted for the vast majority. By comparison, it could be found that the total sugar content of leechee husk crude polysaccharides obtained by UAE way was decidedly surmount that in ASE-LPP, which accounted for 75.65 % of the total crude polysaccharides of UAE-LPP, while the polysaccharides of ASE-LPP obtained by extraction with dilute alkaline solution accounted for 66.23 %. Comparatively, the protein content and total phenol content were also slightly more than that of the alkaline solution extraction method, accounting for 5.68 % and 0.41 % of the total crude polysaccharide content of UAE-LPP, respectively. In contrast, the alkaline solution extraction method was able to extract more uronic acid from the raw material of leechee peel, which was more than twice the amount of uronic acid in UAE-LPP and accounted for 29.28 % of the total crude polysaccharides of ASE-LPP. The total phenolic content of crude polysaccharides in UAE-LPP and ASE-LPP was very small, accounting for 0.41 % and 0.16 %, respectively. In summary, the results showed that UAE was the best method to extract the LPP content from the leechee husk, while the alkaline solution abstraction method was able to get more uronic acid from the leechee husk.

3.5. Structure characterization

3.5.1. Analysis of FT-IR

The infrared spectra of LPP extracted by two different methods were scanned by infrared spectroscopy, and their structures were preliminarily analyzed. The relevant data are shown in Fig. 7. It could be seen that the two polysaccharides, ASE-LPP and UAE-LPP, had strong and broad absorption peaks at 3286.8 cm^{-1} and 3283.9 cm^{-1}separately, which were the expansion and vibration peaks of non-free hydroxyl groups of the polysaccharide chains, indicating that there were

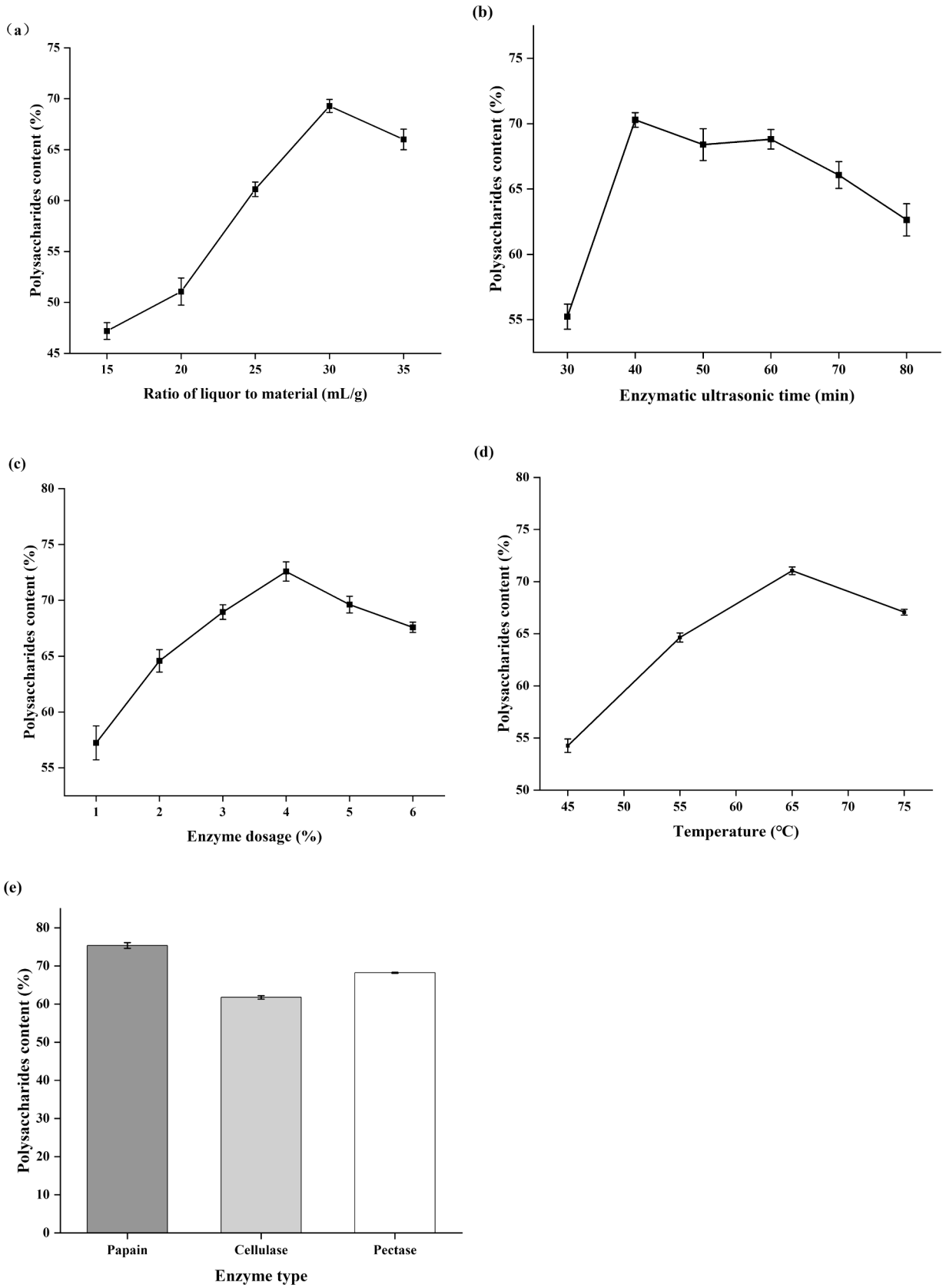

Fig.3. Effects of different factors on UAE of LPP.

Table 8
Response surface experimental design and results.

Serial number	A:Temperature / ℃	B: Ultrasonic time / min	C: Enzyme use / %	R_1: Total sugar content / %
1	55	40	5	68.11
2	55	30	4	66.23
3	65	40	4	79.94
4	55	50	4	65.05
5	75	30	4	59.96
6	65	40	4	80.02
7	65	30	5	72.07
8	65	50	3	70.85
9	65	30	3	73.45
10	65	40	4	79.91
11	65	40	4	78.64
12	55	40	3	67.99
13	65	50	5	71.71
14	75	40	3	60.76
15	75	50	4	60.34
16	65	40	4	79.47
17	75	40	5	61.86

hydrogen bonds in the molecules of these two polysaccharides. The weak absorption peaks at 2929.6 cm^{-1} and 2934.6 cm^{-1} were then caused by C-H asymmetric stretching vibrations. All of them were characteristic absorption peaks of saccharides. The absorption humps at 1599.1 cm^{-1} and 1602.9 cm^{-1} are carboxylate C = O asymmetric stretching vibration peaks. The weak peaks between 1400–1200 cm^{-1} were observed to be variable angle vibrations of C-H. Strong out of peaks appeared near 1012.5 cm^{-1} and 1016.1 cm^{-1}, which originating by the C-O collapsing oscillationon the sugar ring and glycosidic bond, which was the stretching vibration hump of the pyranose ring. These were representative absorption peaks of the characteristic structure of the polysaccharide, from which it was assumed that C-O-H and C-O-C functional groups are present in both samples.

In addition, a weak peak appeared at 1743.6 cm^{-1} for ASE-LPP compared to UAE-LPP. It indicated that there was methyl ester carboxyl group in the sample polysaccharide, which may contain a small amount of uronic acid [48,49]. Nuclear magnetic resonance examination also further confirmed the presence of uronic acid. The characteristic absorption peak at 955.6 cm^{-1} suggested the possible presence of β-type-glycosidic bonds. The characteristic furan ring absorption crests were in the range of 846–758 cm^{-1} with a weak peak at 760.5 cm^{-1}, indicating the presence of a very small amount of furan ring structure in ASE-LPP. A narrow peak at 872.9 cm^{-1} was also observed for UAE-LPP, which may be an α-type glycosidic bond. The functional groups of leechee polysaccharide extracted by ASE and UAE were the same, and the differentiation mainly existed from 1300 to 800 cm^{-1}, and the out of crests in this amplitude mainly reflected that there were some differences in the bonding mode of glycosidic bonds and the composition of monosaccharides in the samples. In summary, the functional group analysis showed that the extracted leechee husk crude polysaccharide conformed to the various functional group characteristic indexes of polysaccharides. The two polysaccharides, ASE-LPP and UAE-LPP, were pyranose cyclic sugars, and the crude polysaccharides of leechee husk obtained by different extraction methods had some structural differences.

3.5.2. Analysis of NMR

In general, the carbon signal peaks of the heterocarbons were mainly concentrated in the range of δ90 to δ110 ppm, with α-heterocarbons between δ90 and δ103 ppm and β-heterocarbons out between δ103 and δ110 ppm. The formant signals of C2-C5 on the Six-membered saccharide ring were located between δ65 and δ80 ppm; whereas, the resonance peak signals of C6 were present in the range of δ60 to δ65 ppm, and the peak signals were shifted to the lower field if C6 was substituted. In this study, one-dimensional nuclear magnetic resonance (1D-NMR) analysis was carried out for leechee husk polysaccharides extracted on the structure by two methods, for which the ^{13}C NMR chemical shifts of the polysaccharides had a wide range of distributions, and therefore the ^{13}C NMR spectra were commonly used to analyze the heteroheads for the polysaccharides.

Fig. 8 (A) showed the spectrum of ^{13}C NMR of UAE-LPP, and it could be seen that the heterocarbon of UAE-LPP was mainly concentrated in the region of δ90 to δ103 ppm, and two strong resonance peak signals, i.e., δ92.23 ppm and δ96.06 ppm, which were attributed to the heterocarbon signals of UAE-LPP in the α-configuration, were appeared. The carbon resonance peaks between δ 68.14 and 75.49 ppm were the C2, C3, C4 and C5 signals on the unsubstituted UAE-LPP sugar ring, respectively. The chemical signals at δ60.79 ppm and δ62.69 ppm were then attributed to C6. In general, chemical shifts that were greater than δ80 ppm were the peak signal areas of furan ring C3 and C5, and conversely, chemical shifts that were less than δ80 ppm were the resonance peak signals of pyran ring C3 and C5. The results showed that in the δ60 ~ 110 ppm region, all signal peaks were smaller than δ80 ppm except for the signal peaks of the heterocarbons, indicating that the UAE-LPP consists of pyran ring only, which coincided with the statistical results about the infrared spectral analysis in 3.4.1 above. In addition, three peak signals were observed in the region of δ160 ~ 180 ppm, which were attributed to the carboxyl carbon signals of glucuronic acid.

The ^{13}C NMR spectrum of ASE-LPP was shown in Fig. 8(B). A resonance peak signal with a chemical shift of 107.52 ppm between δ103 and δ110 ppm was observed, which was attributed to the β-anomeric carbon of ASE-LPP, which was consistent with the results of infrared spectrum analysis. According to the literature, a resonance signal was generated near δ107 ppm and δ82 ppm, indicating that ASE-LPP may contain arabinose. The chemical shift was between 69.45 ~ 76.88 ppm, which was the C2 ~ C5 crests signal of the crude polysaccharide extracted by alkali. At the same time, a weak signal humps with a chemical shift greater than δ80 ppm could be observed in the region of δ60 ~ 110 ppm in addition to the signal peak of the hetero carbon, indicating that perhaps had a small amount of furan ring in UAE-LPP, which was also anastomotic with the result about FT-IR analysis in 3.4.1 above. In addition, a strong signal peak at δ62.14 ppm was the C6 signal of ASE-LPP. In the end, the 13C spectrum of ASE-LPP had a strong resonance signal peak appearing at the chemical shift δ178.63 ppm, which indicated that ASE-LPP contained a certain amount of glucuronic acid, which was consistent with the results of FT-IR and uronic acid content determination.

3.6. Study on the antioxidant activity

Active polysaccharides can exhibit certain antioxidant capacity. In this study, different polysaccharide components (UAE-LPP, ASE-LPP) extracted from leechee peel by different methods showed various antioxidant activities. Three diverse activities under different concentrations of LPP were compared. The results showed that the different polysaccharides extracted from leechee husk showed a certain dose-dependence, i.e., increasing the concentration of polysaccharides used resulted in the enhancement of their antioxidant activity. Therefore, UAE-LPP and ASE-LPP extracted from leechee husk showed some antioxidant activity and are bio-functional polysaccharides. The antioxidant activity of LPP extracted by two types of methods was different. The following showed the antioxidant activities of LPP.

Fig. 9 (A) was the relationship between the scavenging activity of Vc, ASE-LPP and UAE-LPP on hydroxyl radical under a certain concentration gradient. It could be seen that the scavenging effect on ·OH radicals was positively correlated with plycan consistency in 0 ~ 3.0 mg/mL. Of these, the clearance of ·OH by the two polysaccharides was ASE-LPP > UAE-LPP in the order of 31.89 % and 26.67 %, respectively, which had a certain clearance capacity. Nevertheless, the free radical scavenging activity of ASE-LPP and UAE-LPP were lower than that of Vc. LPP crude polysaccharides exhibited relatively low free radical scavenging activity. It had been shown that the hydroxyl radical scavenging effect could

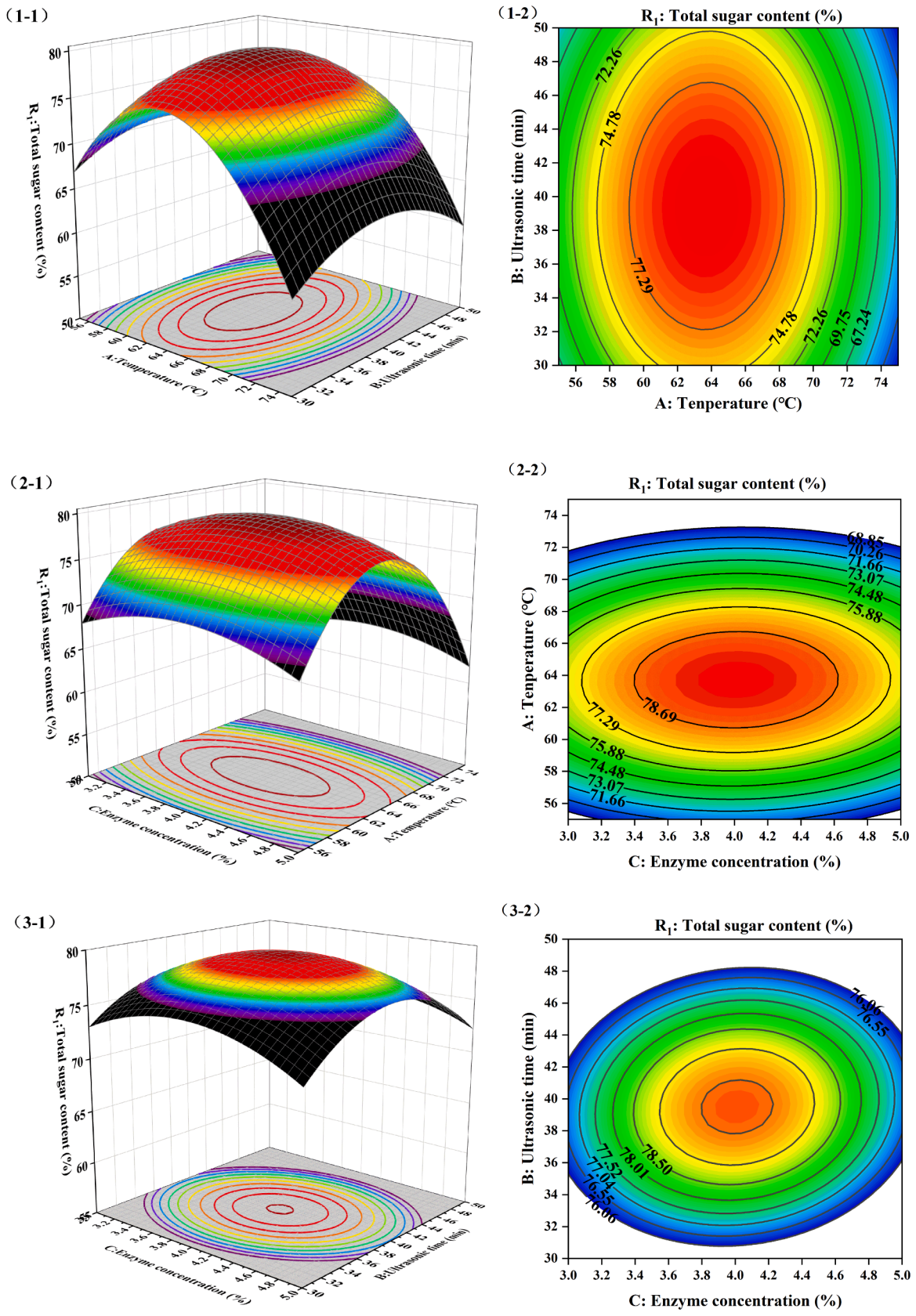

Fig. 4. 3D map and contour T-map of the interaction of factors in the UAE-LPP model.

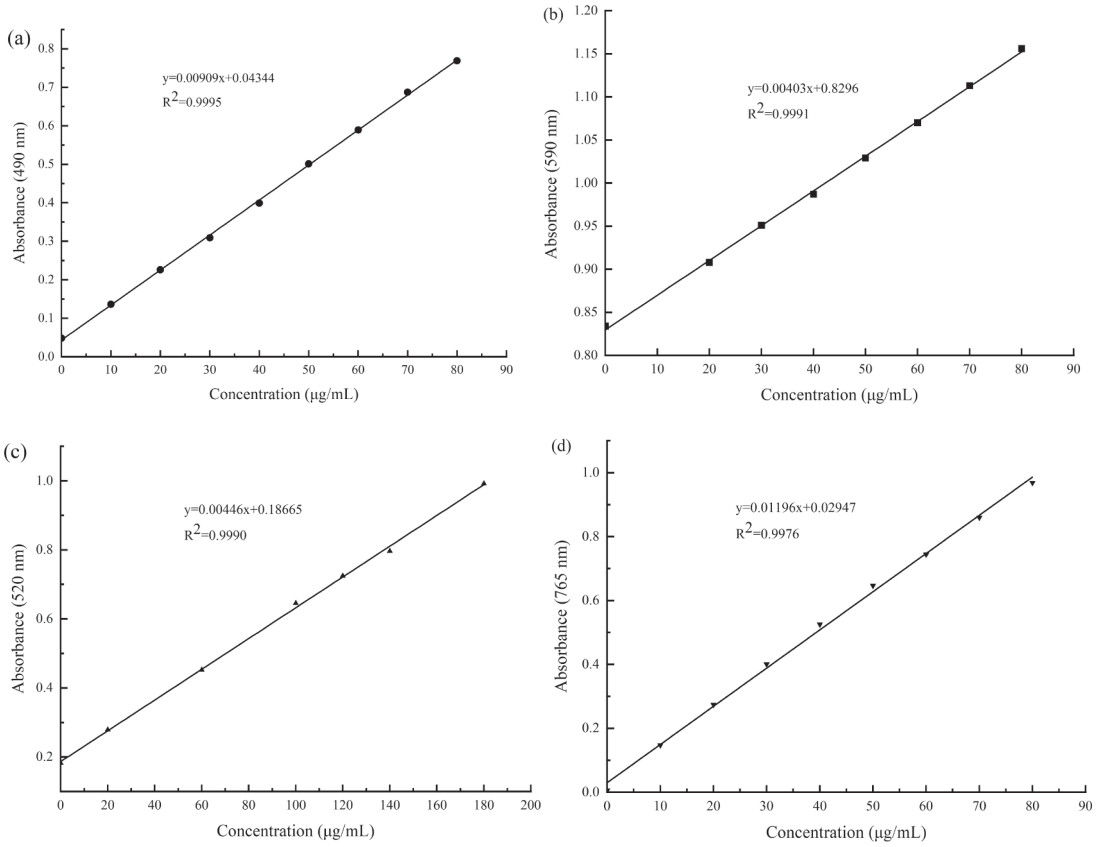

Fig. 5. Standard curve of each component of crude polysaccharide from leechee peel.

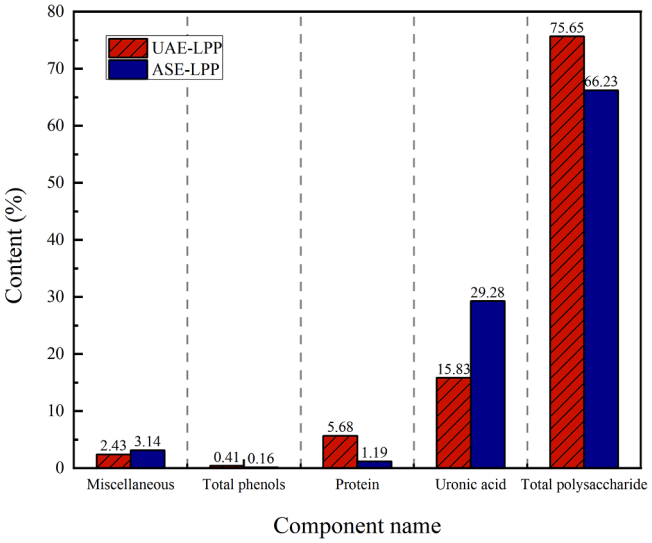

Fig. 6. Basic composition analysis of UAE-LPP and ASE-LPP.

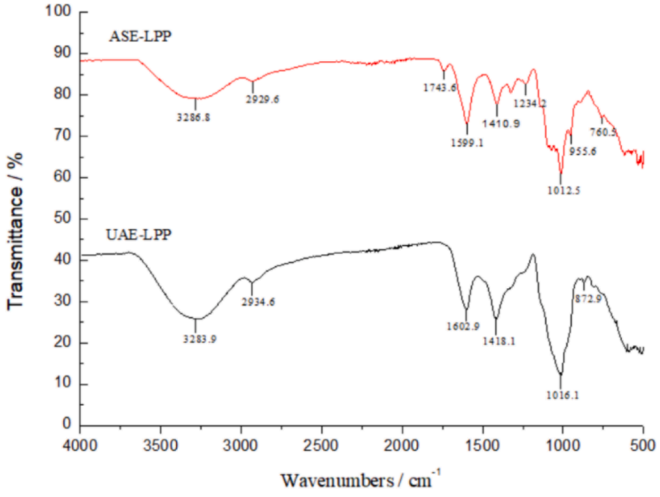

Fig. 7. Infrared spectra of UAE-LPP and ASE-LPP.

be improved by isolation and purification of LPP or the concentration of LPP could be increased as a way to enhance the antioxidant activity. Fig. 9(B) demonstrates the strength of ability of LPP concentration in the 0 ~ 3.0 mg/mL. It could be seen that Vc had good Anti-lipid peroxidation ability (AlP), the AlP ability of ASE-LPP and UAE-LPP increased with the increase of concentration, and in this concentration range, the maximum scavenging rate was 34.51 % and 31.91 %, respectively. And the difference in the maximum scavenging rate was not significant. But in general, the AlP ability of UAE-LPP was stronger than that of ASE-LPP.

As could be seen in Fig. 9(C), both dilute alkaline solution extraction and ultrasonic pectinase method of LPP extraction were positively correlated with reducing power as the concentration of LPP increased. Two polysaccharide reduction capacity was UVE-LPP > ASE-LPP. Among them, the reducing ability of the two polysaccharides was close to each other in the concentration between 1.0 ~ 2.0 mg/mL, and there was a more obvious difference in the reducing ability in the region of 2.0 ~ 3.0 mg/mL. Therefore, it also suggested that reducing ability of LPP obtained by different extraction methods varies, which may be related to the structure of UVE-LPP and ASE-LPP.

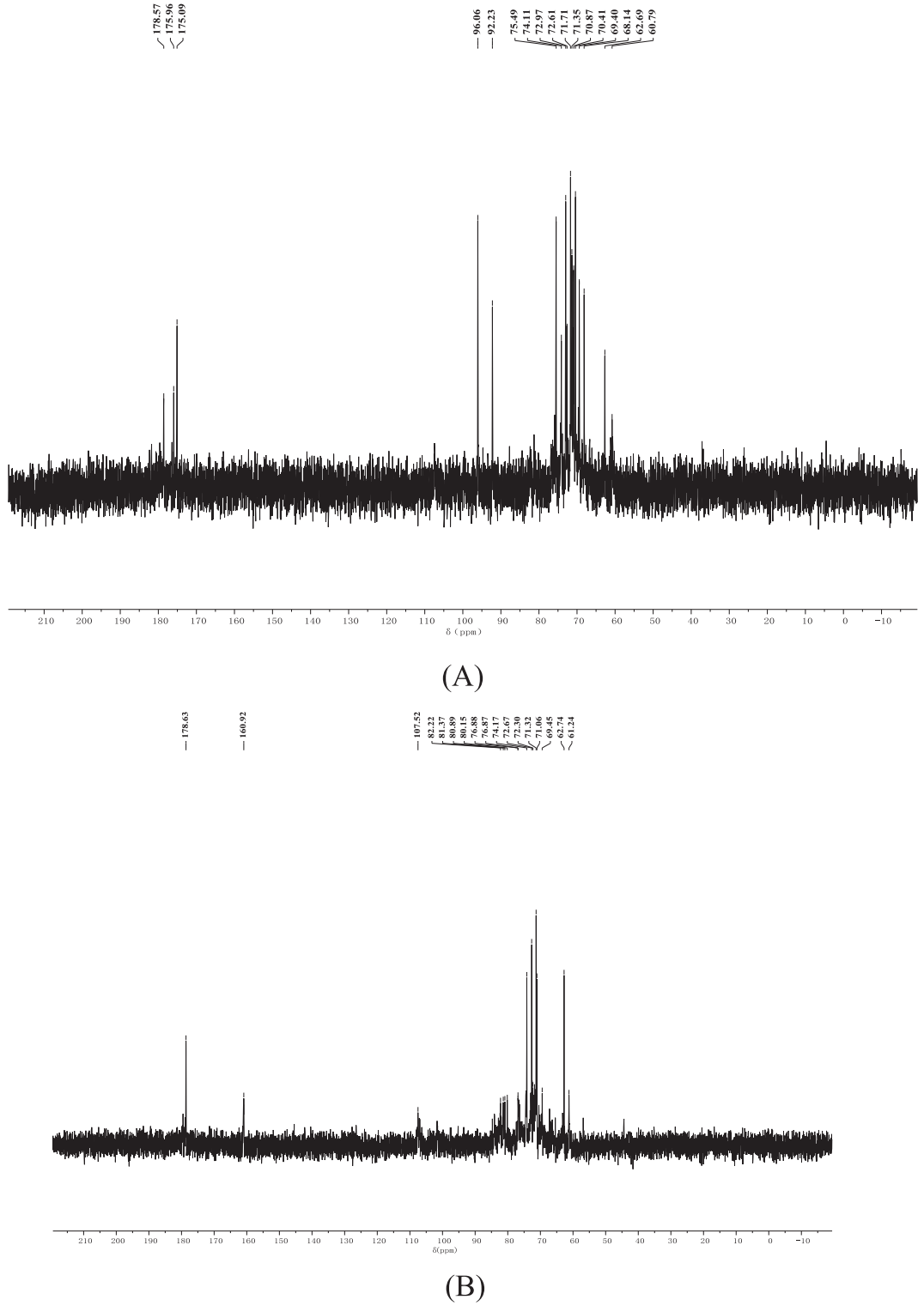

Fig. 8. ^{13}C NMR spectra of UAE-LPP (A) and ASE-LPP (B).

4. Conclusion

This paper used dilute alkali solution extraction and ultrasonic-assisted enzyme extraction of LPP. The two methods were optimized using response surface based on a one-way test to obtain the optimal proceed process: (1) UAE production process: extraction temperature was 68.78 ℃, ultrasound enzyme digestion time was 39.68 min, pectinase dosage was 4.03 %, the material-water ratio was 1:30 g/mL, and the ultrasound power was 280 W. The total sugar content of UAE-LPP was measured as 75.65 % by UV. (2) Dilute alkaline solution extraction method: concentration of sodium was 3.18 %, time-consuming was 90.15 min, holding temperature was 59.87 °C, material-water ratio was 1:25 g/mL; total sugar content in ASE-LPP was 66.23 %. The goodness of fit with the theoretical values implies that the mould gain had high reliability and accuracy in this investigation. By optimizing the two extraction methods, the contents of polysaccharide and uronic acid were

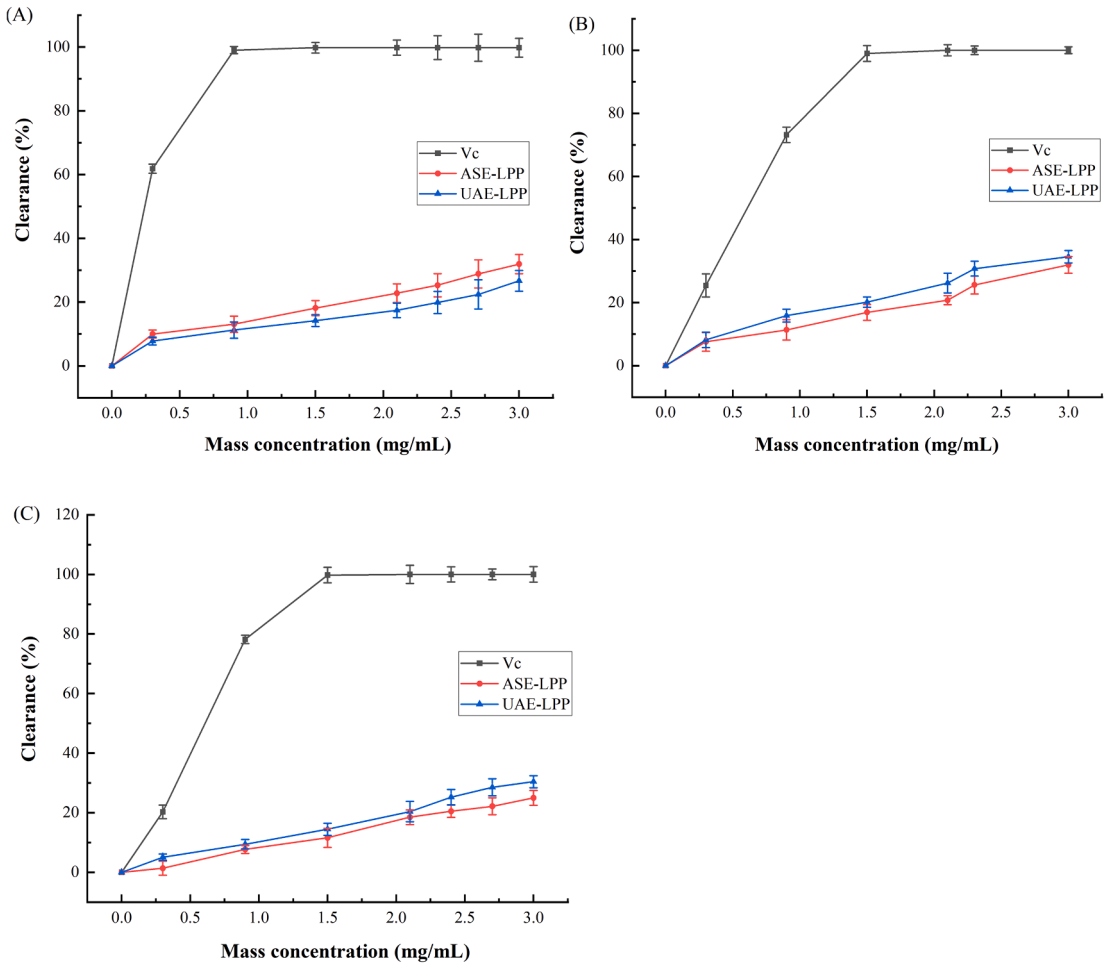

Fig. 9. Comparative study on antioxidant activity.

increased, and the utilization rate of leechee peel was greatly improved. FT-IR and NMR modern techniques were used to confirm that the two polysaccharides were different in structure. UAE-LPP was composed of α-pyran ring, ASE-LPP was composed of β-pyran ring, and there was also a small amount of furan ring structure. By comparing the antioxidant capacity of UAE-LPP and ASE-LPP, the anti-lipid peroxidation ability and reducing ability of UAE-LPP were stronger than those of ASE-LPP.

This study provides a theoretical basis and technical support for the further development and utilization of LPP, but there is no comparative study on different varieties of leechee peel, and the structure of LPP is relatively simple. In the future, the structure–activity relationship is one of the main directions of polysaccharide research. Uncovering the mystery of the high-level structure of polysaccharides, in order to reveal the medicinal principle and other value of LPP, and open up a broader space for the application of leechee peel.

CRediT authorship contribution statement

Yijie Wang wrote the manuscript. Gangliang Huang and Hualiang Huang reviewed & edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S. Punia, M. Kumar, Litchi (Litchi chinenis) seed: Nutritional profile, bioactivities, and its industrial applications, Trends Food Sci. Technol. 108 (2021) 58–70, https://doi.org/10.1016/j.tifs.2020.12.005.
[2] S. Xu, H. Lu, X. Sun, Quality detection of postharvest leechee based on electronic nose: a feasible way for leechee fruit supervision during circulation process, HortSci. 55 (2020) 476–482, https://doi.org/10.21273/HORTSCI14750-19.
[3] L. Zhao, K. Wang, K. Wang, J. Zhu, Z. Hu, Nutrient components, health benefits, and safety of litchi (*Litchi chinensis* Sonn.): a review, Compr. Rev. Food Sci. Food Safety. 19 (2020) 2139–2163, https://doi.org/10.1111/1541-4337.12590.
[4] M. Ortiz-Sanchez, C.A. Cardona Alzate, J.C. Solarte-Toro, Orange peel waste as a source of bioactive compounds and valuable products: insights based on chemical composition and biorefining, Biomass 4 (2024) 107–131, https://doi.org/10.3390/biomass4010006.
[5] S. Manzoor, A. Rakha, H. Rasheed, S. Munir, G. Abdi, Z.F. Bhat, R.M. Aadil, Development and evaluation of anxiolytic potential of bagels incorporated with banana peel flour and lavender, J. Agric. Food Res. 15 (2024) 101029, https://doi.org/10.1016/j.jafr.2024.101029.
[6] F. Azmat, M. Safdar, H. Ahmad, M.R. Khan, J. Abid, M.S. Naseer, S. Aggarwal, A. Imran, U. Khalid, S.M. Zahra, F. Islam, Phytochemical profile, nutritional composition of pomegranate peel and peel extract as a potential source of nutraceutical: a comprehensive review, Food Sci. Nutr. 12 (2024) 661–674, https://doi.org/10.1002/fsn3.3777.
[7] Y. Chen, F. Yao, K. Ming, D. Wang, Y. Hu, J. Liu, Polysaccharides from traditional Chinese medicines: extraction, purification, modification, and biological activity, Molecules 21 (2016) 1705, https://doi.org/10.3390/molecules21121705.
[8] S. Tang, N. Luo, Q. Zeng, L. Dong, R. Zhang, S. He, A. Nag, F. Huang, D. Su, Lychee pulp phenolics fermented by mixed lactic acid bacteria strains promote the metabolism of human gut microbiota fermentation *in vitro*, Food Funct. 14 (2023) 7672–7681, https://doi.org/10.1039/D3FO01668D.
[9] Y. Chen, H. Li, S. Zhang, C. Yang, Z. Mai, X. Hu, Z. Gao, H. Deng, Anti-myocardial ischemia effect and components of litchi pericarp extracts, Phytother. Res. (2017) 1384–1391, https://doi.org/10.1002/ptr.5865.
[10] Y. Liao, Z. Luo, Y. Liu, W. Xue, S. He, X. Chen, H. Ren, X. Yang, D. Zhu, Z. Su, Q. Huang, Total flavonoids of Litchi seed attenuate stem cell-like properties in

breast cancer by regulating Notch3 signaling pathway, J. Ethnopharmacol. 305 (2023) 116133, https://doi.org/10.1016/j.jep.2023.116133.
[11] B.G. Nabi, K. Mukhtar, S. Ansar, S.A. Hassan, M.A. Hafeez, Z.F. Bhat, A. M. Khaneghah, A.U. Haq, R.M. Aadil, Application of ultrasound technology for the effective management of waste from fruit and vegetable, Ultrason. Sonochem. 102 (2024) 106744, https://doi.org/10.1016/j.ultsonch.2023.106744.
[12] B. Lin, S. Wang, A. Zhou, Q. Hu, G. Huang, Ultrasound-assisted enzyme extraction and properties of Shatian pomelo peel polysaccharide, Ultrason. Sonochem. 98 (2023) 106507, https://doi.org/10.1016/j.ultsonch.2023.106507.
[13] C. Teng, P. Qin, Z. Shi, W. Zhang, X. Yang, Y. Yao, G. Ren, Structural characterization and antioxidant activity of alkali-extracted polysaccharides from quinoa, Food Hydrocolloid. 113 (2021) 106392, https://doi.org/10.1016/j.foodhyd.2020.106392.
[14] Y. Wang, X. Zhang, X. Ma, K. Zhang, S. Li, X. Wang, X. Liu, J. Liu, W. Fan, Y. Li, Q. Li, Study on the kinetic model, thermodynamic and physicochemical properties of Glycyrrhiza polysaccharide by ultrasonic assisted extraction, Ultrason. Sonochem. 51 (2019) 249–257, https://doi.org/10.1016/j.ultsonch.2018.10.012.
[15] S. Zhou, A. Rahman, J. Li, C. Wei, J. Chen, R.J. Linhardt, X. Ye, S. Chen, Extraction methods affect the structure of Goji (Lycium barbarum) polysaccharides, Molecules 25 (2024) 936, https://doi.org/10.3390/molecules25040936.
[16] W. Zhang, W. Duan, G. Huang, H. Huang, Ultrasonic-assisted extraction, analysis and properties of mung bean peel polysaccharide, Ultrason. Sonochem. 98 (2023) 106487, https://doi.org/10.1016/j.ultsonch.2023.106487.
[17] Y. Fan, G. Huang, Preparation and analysis of *Pueraria lobata* polysaccharides, ACS Biomater. Sci. Eng. 9 (2023) 2329–2334, https://doi.org/10.1021/acsbiomaterials.2c01479.
[18] W. Yang, G. Huang, Preparation and analysis of polysaccharide from Solanum tUAErdsm, Ultrason. Sonochem. 98 (2023) 106520, https://doi.org/10.1016/j.ultsonch.2023.106520.
[19] Z. Tang, G. Huang, H. Huang, Ultrasonic/cellulase-assisted extraction of polysaccharide from *Garcinia mangostana* rinds and its carboxymethylated derivative, Ultrason. Sonochem. 99 (2023) 106571, https://doi.org/10.1016/j.ultsonch.2023.106571.
[20] L. He, X. Yan, J. Liang, S. Li, H. He, X. Lai, S. Hou, S. Huang, Comparison of different extraction methods for polysaccharides from Dendrobium officinale stem, Carbohydr. Polym. 198 (2018) 101–108, https://doi.org/10.1016/j.carbpol.2018.06.073.
[21] J. Ai, Z. Yang, J. Liu, H.A. Schols, M. Battino, B. Bao, L. Tian, W. Bai, Structural characterization and in vitro fermentation characteristics of enzymatically extracted black mulberry polysaccharides, J. Agric. Food Chem. 70 (2022) 3654–3665, https://doi.org/10.1021/acs.jafc.1c07810.
[22] I.F. Olawuyi, S.R. Kim, D. Hahn, W.Y. Lee, Influences of combined enzyme-ultrasonic extraction on the physicochemical characteristics and properties of okra polysaccharides, Food Hydrocolloid. 100 (2020) 105396, https://doi.org/10.1016/j.foodhyd.2019.105396.
[23] X. Hu, F. Xu, J. Li, J. Li, C. Mo, M. Zhao, L. Wang, Ultrasonic-assisted extraction of polysaccharides from coix seeds: optimization, purification, and in vitro digestibility, Food Chem. 374 (2022) 131636, https://doi.org/10.1016/j.foodchem.2021.131636.
[24] M. Wang, C. Zhang, Y. Xu, M. Ma, T. Yao, Z. Sui, Impact of six extraction methods on molecular composition and antioxidant activity of polysaccharides from young hulless barley leaves, Foods. 12 (2023) 3381, https://doi.org/10.3390/foods12183381.
[25] Y.Y. Jiang, J. Yu, Y.B. Li, L. Wang, L. Hu, L. Zhang, Y.H. Zhou, Extraction and antioxidant activities of polysaccharides from roots of Arctium lappa L, Int. J. Biol. Macromol. 123 (2019) 531–538, https://doi.org/10.1016/j.ijbiomac.2018.11.087.
[26] F. Liu, H. Chen, L. Qin, A.A. Al-Haimi, J. Xu, W. Zhou, S. Zhu, Z. Wang, Effect and characterization of polysaccharides extracted from Chlorella sp. by hot-water and alkali extraction methods, Algal Res. 70 (2023) 102970, https://doi.org/10.1016/j.algal.2023.102970.
[27] Y. Sun, S. Hou, S. Song, B. Zhang, C. Ai, X. Chen, N. Liu, Impact of acidic, water and alkaline extraction on structural features, antioxidant activities of Laminaria japonica polysaccharides, Int. J. Biol. Macromol. 112 (2018) 985–995, https://doi.org/10.1016/j.ijbiomac.2018.02.066.
[28] X. Lin, X. Ji, M. Wang, S. Yin, Q. Peng, An alkali-extracted polysaccharide from *Zizyphus jujuba* cv. Muzao: structural characterizations and antioxidant activities, Int. J. Biol. Macromol. 136 (2019) 607–615, https://doi.org/10.1016/j.ijbiomac.2019.06.117.
[29] F. Yue, J. Zhang, J. Xu, T. Niu, X. Lü, M. Liu, Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method, Front. Nutr. 9 (2022) 963318, https://doi.org/10.3389/fnut.2022.963318.
[30] F. Chen, G. Huang, Antioxidant activity of polysaccharides from different sources of ginseng, Int. J. Biol. Macromol. 125 (2019) 906–908, https://doi.org/10.1016/j.ijbiomac.2018.12.134.
[31] X. Xong, W. Yang, G. Huang, H. Huang, Ultrasonic-assisted extraction, characteristics and activity of Ipomoea batatas polysaccharide, Ultrason. Sonochem. 96 (2023) 106420, https://doi.org/10.1016/j.ultsonch.2023.106420.
[32] K. Grintzalis, C.D. Georgiou, Y.J. Schneider, An accurate and sensitive Coomassie Brilliant Blue G-250-based assay for protein determination, Anal. Biochem. 480 (2015) 28–30, https://doi.org/10.1016/j.ab.2015.03.024.
[33] J.I. Dacheng, G.A. Shan, G.A. Hailun, Q.I. Nianwei, The details of protein content determination by Coomassie brilliant blue staining, Exp. Sci. Technol. 16 (2018) 143–147, https://doi.org/10.3969/j.issn.1672-4550.2018.04.35.
[34] S.G. Shen, S.R. Jia, Y.K. Wu, R.R. Yan, Y.H. Lin, D.X. Zhao, P.P. Han, Effect of culture conditions on the physicochemical properties and antioxidant activities of polysaccharides from Nostoc flagelliforme, Carbohydr. Polym. 198 (2018) 426–433, https://doi.org/10.1016/j.carbpol.2018.06.111.
[35] Y. Yu, H. Li, B. Zhang, J. Wang, X. Shi, J. Huang, J. Yang, Y. Zhang, Z. Deng, Nutritional and functional components of mulberry leaves from different varieties: evaluation of their potential as food materials, Int. J. Food Prop. 21 (2018) 1495–1507, https://doi.org/10.1080/10942912.2018.1489833.
[36] M. Platzer, S. Kiese, T. Herfellner, U. Schweiggert-Weisz, P. Eisner, How does the phenol structure influence the results of the Folin-Ciocalteu assay? Antioxidants. 10 (2021) 811, https://doi.org/10.3390/antiox10050811.
[37] J.Q. Wang, J.Y. Yin, S.P. Nie, A review of NMR analysis in polysaccharide structure and conformation: progress, challenge and perspective, Food Res. Int. 143 (2021) 110290, https://doi.org/10.1016/j.foodres.2021.110290.
[38] Y. Liu, Y. Sun, G. Huang, Preparation and antioxidant activities of important traditional plant polysaccharides, Int. J. Biol. Macromol. 111 (2018) 780–786, https://doi.org/10.1016/j.ijbiomac.2018.01.086.
[39] M.M. Ahmad, S.A. Chatha, Y. Iqbal, A.I. Hussain, I. Khan, Recent trends in extraction, purification, and antioxidant activity evaluation of plant leaf-extract polysaccharides, Biofuel. Bioprod. Bior. 16 (2022) 1820–1848, https://doi.org/10.1002/bbb.2405.
[40] S. Zhou, H. Zou, G. Huang, G. Chen, Preparations and antioxidant activities of sesamol and it's derivatives, Bioorg. Med. Chem. Lett. 31 (2021) 127716, https://doi.org/10.1016/j.bmcl.2020.127716.
[41] S. Wang, G. Li, X. Zhang, Y. Wang, Y. Qiang, B. Wang, J. Zou, J. Niu, Z. Wang, Structural characterization and antioxidant activity of Polygonatum sibiricum polysaccharides, Carbohydr. Polym. 291 (2022) 119524, https://doi.org/10.1016/j.carbpol.2022.119524.
[42] Z. Tang, Y. Wang, G. Huang, H. Huang, Ultrasound-assisted extraction, analysis and antioxidant activity of polysaccharide from the rinds of *Garcinia mangostana* L, Ultrason. Sonochem. 97 (2023) 106474, https://doi.org/10.1016/j.ultsonch.2023.106474.
[43] R. Hashemifesharaki, E. Xanthakis, Z. Altintas, Y. Guo, S.M. Gharibzahedi, Microwave-assisted extraction of polysaccharides from the marshmallow roots: optimization, purification, structure, and bioactivity, Carbohydr. Polym. 240 (2020) 116301, https://doi.org/10.1016/j.carbpol.2020.116301.
[44] W. Song, Z. Xu, P. Gao, X. Liu, Chemical composition and in vitro antioxidant activity and anti-acetylcholinesterase activity of essential oils from Tadehagi triquetrum (L.) ohashi, Molecules 28 (2023) 2734, https://doi.org/10.3390/molecules28062734.
[45] X. Hu, J. Wang, L. Song, J. Zhu, X. Cui, R. Yu, Structural elucidation and in vitro antioxidant activities of a new heteropolysaccharide from Litchi chinensis, Drug Discov. Ther. 9 (2015) 116–122, https://doi.org/10.5582/ddt.2015.01022.
[46] W. Gao, P. Lin, X.A. Zeng, M.A. Brennan, Preparation, characterisation and antioxidant activities of litchi (Litchi chinensis Sonn.) polysaccharides extracted by ultra-high pressure, Int. J. Food Sci. Technol. 52 (2017) 1739–1750, https://doi.org/10.1111/ijfs.13447.
[47] Y.S. Hsieh, C.Y. Wong, The composition of polysaccharides in primary walls of Litchi chinensis Sonn, J. Food Biochem. 34 (2010) 971–982, https://doi.org/10.1111/j.1745-4514.2010.00339.x.
[48] L. Lin, M. Zhuang, L. Zou, F. Lei, B. Yang, M. Zhao, Structural characteristics of water-soluble polysaccharides from Rabdosia serra (MAXIM.) HARA leaf and stem and their antioxidant capacities, Food Chem. 135 (2012) 730–737, https://doi.org/10.1016/j.foodchem.2012.05.029.
[49] H. Zhang, H. Zhao, X. Zhou, X. Yang, S. Shen, J. Wang, Z. Wang, L. Geng, Isolation and characterization of antioxidant polysaccharides (PKCP-D 70–2-a and PKCP-D 70–2-b) from the Pinus koraiensis pinecone, RSC Adv. 6 (2016) 110706–110721, https://doi.org/10.1039/C6RA23205A.