Extraction of Low Methoxyl Pectin from Fresh Sunflower Heads by Subcritical Water Extraction

Xuemei Ma, Jing Jing, Jingbao Wang, Jingjing Xu, and Zhiyong Hu

ABSTRACT: Subcritical water extraction (SWE) of pectin from fresh sunflower heads was optimized using the response surface methodology (RSM). The optimal conditions for the maximum yield of pectin (6.57 ± 0.6%) were found to be a pressure of 8 bar, temperature of 120 °C, time of 20 min, and liquid–solid ratio (LSR) of 7 mL/g. The degree of esterification (DE) of pectin was analyzed by titrimetry and Fourier transform infrared (FTIR) methods, which was low methoxyl pectin. The molecular weight (Mw), galacturonic acid (GalA) content, and surface tension of pectin were 11.50 kDa, 82%, and 45.38 mN/m (1.5% w/v), respectively. Moreover, thermogravimetric (TG) and differential scanning calorimetry (DSC) analysis confirmed that pectin had excellent thermal stability. FTIR and 1H NMR spectra confirmed its structure. This study demonstrated that SWE could be used as a productive and environmentally friendly method for extracting pectin from fresh sunflower heads.

1. INTRODUCTION

Pectin can be composed of as many as 17 different monosaccharides that contain more than 20 different linkages.1 The primary structural components of pectin include homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). HG is a linear polymer composed of a 1,4-linked α-D-galacturonic acid (α-D-GalA) backbone. RG-I comprises the repeating disaccharide [-4)-α-D-GalA-(1−2)-α-L-Rha(1−], whereas RG-II has a backbone of HG with complex side chains attached to the GalA residues.2 Low methoxyl pectin (LMP) with a degree of esterification (DE) lower than 50% has recently attracted considerable attention because of its applications in functional foods and pharmaceuticals.3 LMP is not only highly suitable for wound-healing applications but also has good compatibility with yogurt; moreover, it could be widely used as a carrier for delivering probiotics.4,5 Furthermore, LMP can be used as a thickening agent in acidic dairy products and can reinforce the firmness of Japonica rice noodles when Ca2+ is added to the mixture.6,7 Moreover, LMP gels can be used in low-calorie jams and jellies for glazing, retorting, microwaving, baking, and sterilizing or pasteurizing.8 Typically, LMP is obtained from natural sources or prepared from high methoxyl pectin; however, synthetically preparing LMP from high methoxyl pectin is expensive.9,10 Nevertheless, the yield and quality of pectin are affected by raw materials, stage of maturity, and extraction conditions.11 Note that sunflower heads, which are discarded during sunflower seed harvest, contain pectin that is naturally low-methoxylated. Because the heads are rich in pectin and contain 18–24% pectin after seed removal, they have been suggested as an excellent natural source of LMP.10 Traditionally, pectin can be extracted in multiple ways using hot water, dilute alkali solutions, salt solutions, enzymes, and acidic solutions.11−15 Previous studies on pectin extraction from sunflower heads employed dried mature sunflower heads as the raw material. The extracted pectin was tanned or black in color because of the presence of brown water-soluble pigments; however, commercial pectin products are expected to be a light colored or a colorless powder. The amount of pectin, composition, and physicochemical properties can be considerably affected by the decolorization step. Therefore, pigment removal is critical to the quality of pectin and has an impact on the overall cost. Furthermore, the current extraction methods have certain disadvantages such as being unsafe, expensive to operate, and can generate considerable environmental pollution.16−18 To address these limitations of LMP extraction methods, we utilized fresh sunflower heads as the raw material and looked for innovative extraction techniques. Subcritical water extraction (SWE) is a well-known environmentally friendly and...
green method for extracting natural materials. Subcritical water is water maintained in the liquid state at temperatures between 100 and 374 °C under pressure. In this state, the dielectric constant and polarity of water can be changed. Because water is nontoxic, inexpensive, readily available, and can be easily disposed, using subcritical water for extraction can be both cost-effective and environmentally friendly. To date, most of the SWE studies on pectin have used extraction pressures and extraction liquid–solid ratios (LSRs) in the range of 20–180 bar and 10−70 mL/g, respectively. In this study, we studied low extraction pressures to improve the production safety and smaller extraction LSRs to investigate the possibility of decreasing the amount of water required without affecting pectin loss. Experimental design (Box–Behnken design (BBD) in this work) is used to reduce the number of assays needed for optimizing conditions while the response surface methodology (RSM) is used to analyze the results of the experimental design.

To our knowledge, the extraction of pectin from fresh sunflower heads by subcritical water technology has not been reported to date. In this study, we aimed to develop an optimized, ecofriendly, SWE protocol for obtaining pectin from fresh sunflower heads. Further, the fresh sunflower heads pectin (SFHP) was also characterized using Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (1H NMR), the molecular weight ($M_w$) was determined using high-performance size exclusion chromatography (HPSEC), and thermal properties were evaluated using thermogravimetric (TG) and differential scanning calorimetry (DSC) analysis. The DE of pectin was also determined by both titrimenty and FTIR methodology, and we expect that SFHP will be well received by the food industry as a stabilizing agent.

2. RESULTS AND DISCUSSION

2.1. Single-Factor Analysis for the Extraction of SFHP

2.1.1. Effect of Extraction Time on SFHP Yield. The influence of extraction time on SFHP yield was reported in this study. The effect of extraction time (10−50 min) on pectin yield was examined by fixing an LSR of 2 mL/g, pressure of 10 bar, and extraction temperature of 140 °C. As shown in Figure 1a, the extraction yield of SFHP increased with increase in extraction time to the peak value (5.58%) when the extraction time was 20 min. No increase in the yield above the peak value occurred from 20 to 50 min. The appropriate extraction time facilitated...
the mass transfer of pectin from plant tissues into solution. The cell wall was broken, and pectin was released. However, prolonged extraction time decreased the yield because of pectin decomposition.25 Therefore, in this study, we used 20 min as the time interval for all BBD experiments (Section 2.2).

2.1.2. Effect of Pressure on SFHP Yield. The effect of extraction pressure (2–10 bar) on the yield of SFHP was examined by fixing the LSR to 2 mL/g, temperature to 140 °C, and extraction time to 30 min. The SFHP yields increased with the increase of pressure from 2 to 8 bar (Figure 1b). The plant material was heated in pressure, the cell structure tended to break down, and insoluble pectin was hydrolyzed into soluble pectin. Therefore, pectin yields increased. The pressure increased, the surface tension of water decreased, and the intensity of water increased, which was beneficial for pectin extraction. At pressures above 8 bar, no di

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Table 1. Experimental Values for the Yield of SFHP from the BBD Experimental Design

| run | pressure (bar) | temperature (°C) | LSR (mL/g) | yield (%) |
|-----|----------------|------------------|------------|-----------|
| 1   | 8              | 100              | 4          | 2.54      |
| 2   | 8              | 140              | 4          | 1.61      |
| 3   | 10             | 120              | 4          | 2.15      |
| 4   | 10             | 100              | 6          | 3.66      |
| 5   | 10             | 120              | 8          | 4.64      |
| 6   | 6              | 120              | 8          | 2.24      |
| 7   | 8              | 120              | 6          | 7.06      |
| 8   | 8              | 140              | 8          | 2.73      |
| 9   | 8              | 120              | 6          | 6.44      |
| 10  | 6              | 100              | 6          | 3.58      |
| 11  | 8              | 120              | 6          | 6.84      |
| 12  | 10             | 140              | 6          | 3.33      |
| 13  | 8              | 100              | 8          | 3.46      |
| 14  | 6              | 140              | 8          | 2.66      |
| 15  | 6              | 120              | 4          | 2.76      |

second-order equations of the extraction yield are expressed as follows

\[Y = 6.78 + 0.32X_1 - 0.36X_2 + 0.50X_3 + 0.15X_1X_2 + 0.75X_2X_3 + 0.05X_2X_3 - 1.55X_1^2 - 1.92X_2^2 - 2.28X_3^2\]  

where \(Y\) is the yield of pectin and \(X_i\) represents the coded variable (\(X_1\) is the pressure, \(X_2\) is the temperature, and \(X_3\) is the LSR).

The analysis of variance (ANOVA) results are presented in Table 2. The corresponding variables would be more significant if the \(F\)-value became greater and the \(p\)-value became smaller. As shown in Table 2, the \(p\)-value of the model was 0.0001, indicating that the model was significant. The \(F\)-value was 66.69, indicating that there was only a 0.01% chance that this could occur because of the noise in the model. The “lack of fit” \(F\)-value of 0.16 indicated that the lack of fit was not significant relative to pure error. The quadratic regression model showed that the value of the determination coefficient (\(R^2\)) was 0.99, which indicated that 99% of the variation could be explained by the model. For a good statistical model, \(adj R^2\) should be close to \(R^2\). As shown in Table 2, \(adj R^2\) was 0.98, which indicated that only 2% of the total variation was not explained by the model. The “pred \(R^2\)” of 0.93 was in reasonable agreement with the “\(adj R^2\)” of 0.98; moreover, a relatively low coefficient of variation value (3.64%) indicated good reliability of the experimental values. From the above
The response surfaces provided a method to visualize the relationship between the responses and the independent variables. In this work, response surfaces were obtained by maintaining one of the variables constant at a zero level while varying the other two variables. The response surface plot showed the magnitude of the response values. As shown in Figure 2, when other variables were fixed at a zero level, the slope of the response surface was relatively flat, which suggested that the extraction conditions had little effect on the yield. However, if the slope was relatively steep, it indicated that the extraction conditions considerably affected the yield.21 Figure 2 showed that the extraction LSR played an important role in SFHP yield. Furthermore, the shape of contour plots reflected the extent of the interaction effect: an ellipse showed a significant interaction effect between factors, whereas a circle showed no significant impact. The maximum response value was obtained at the center of the ellipse-shaped region and gradually decreased from the center to the edge.

Based on the response surface and contour plots, it was confirmed that moderate pressure, temperature, and LSR conditions would increase the pectin yield. Thus, we determined that the effect on the SFHP was reduced in the following order \( X_3 > X_2 > X_1 \) according to the \( p \)-value. Based on the BBD-derived model, the recommended extraction conditions for the maximum SFHP yield were as follows:

**Table 2. Analysis of Variance (ANOVA) for the BBD Model**

| source      | sum of squares | DF | mean square | \( F \)-value | \( p \)-value |
|-------------|----------------|----|-------------|---------------|--------------|
| model       | 42.55          | 9  | 4.73        | 66.69         | 0.0001       |
| \( X_1 \) — pressure | 0.81          | 1  | 0.81        | 11.38         | 0.0198       |
| \( X_2 \) — temp      | 1.06          | 1  | 1.06        | 14.93         | 0.0118       |
| \( X_3 \) — LSR        | 2.01          | 1  | 2.01        | 28.36         | 0.0031       |
| \( X_1 \) \( X_2 \) | 0.09          | 1  | 0.087       | 1.23          | 0.3183       |
| \( X_1 \) \( X_3 \) | 2.27          | 1  | 2.27        | 31.95         | 0.0024       |
| \( X_2 \) \( X_3 \) | 0.01          | 1  | 0.01        | 0.14          | 0.7226       |
| \( X_1 \)^2         | 8.93          | 1  | 8.93        | 125.95        | <0.0001      |
| \( X_2 \)^2         | 13.58         | 1  | 13.58       | 191.52        | <0.0001      |
| \( X_3 \)^2         | 19.15         | 1  | 19.15       | 270.18        | <0.0001      |
| residual             | 0.35          | 5  | 0.02        |               |              |
| lack of fit          | 0.16          | 3  | 0.05        | 0.53          | 0.7057       |
| pure                  | 0.20          | 2  | 0.10        |               |              |
| cor total            | 42.9          | 14 |              |               |              |
| \( X \)              | 0.99          |    |             |               |              |
| \( \text{adj } R^2 \) | 0.98          |    |             |               |              |
| \( \text{pred } R^2 \) | 0.93          |    |             |               |              |

Figure 2. Response surface and contour plots showing the effects of independent variables on the yield of SFHP. (a) Temperature vs pressure, (b) LSR vs pressure, and (c) LSR vs temperature.
pressure of 8.3 bar, temperature of 120 °C, and LSR of 6.72 mL/g. Finally, an extraction run with slightly sub-optimal reaction conditions for SFHP yield (pressure of 8 bar, temperature of 120 °C, and LSR of 7 mL/g) was completed. At these conditions, the predicted yield was 6.85%, with a desirability value of 0.99. To confirm and ensure that the predicted values did not considerably deviate from the true experimental values, extractions were performed in triplicate using the slightly modified predicted optimal extraction conditions, and then an average pectin yield of 6.57 ± 0.6% was obtained. This value was in very good agreement with the model-predicted value, indicating that the model could be used safely for optimizing the extraction of SFHP.

2.3. Determination of the Molecular Weight. The functional properties of pectin significantly depend on the $M_w$. The $M_w$ values of SFHP extracted by sodium citrate and ammonium oxalate from dried sunflower heads were 256.40 and 605.60 kDa, respectively. For extraction of pectin by sodium hexametaphosphate from dried sunflower heads, the $M_w$ ranged from 39 to 52 kDa. Pectin was extracted from pistachio green hull with the $M_w$ of 1.65 kDa. In this study, the $M_w$ was 11.5 kDa. Therefore, the SFHP had a reasonable $M_w$. Moreover, in our previous study, the $M_w$ value of pectin was affected by the drying process of the raw material. On the one hand, the pectin in fresh sunflower heads may have a low $M_w$ related to the stage of maturity and the moisture content of the raw material. On the other hand, the low observed $M_w$ under SWE may be because of pectin hydrolysis and decomposition under subcritical water temperature and pressure. When comparing SWE and other extraction methods, the lower $M_w$ values were obtained from SWE methods. Certainly, these values agreed with the $M_w$ range of 8–1000 kDa for the pectin extracted from miscellaneous fruit sources. Moreover, the $M_w$/ $M_n$ of 2.49 was much higher than 1, indicating that SFHP had a wide $M_w$ distribution and was a heterogeneous natural polysaccharide. Pectin from the same source could have different structural differences because of different extraction methods and storage times, which further affected its functionality. For a fixed raw material, the extraction condition was the most important factor. Therefore, it was possible to produce pectin with specific characteristics using different methods and widen its application potential.

2.4. Physicochemical Properties of SFHP. The DE and chemical parameters are listed in Tables 3 and 4, respectively.

| Table 3. Comparison of Titrimetry and FTIR Methods on the DE of SFHP Extracted at Optimized Conditions |
|---------------------------------------------------|
| method | DE (%) |
| titrimetry | 19.4 ± 0.95 |
| FTIR | 17.7 ± 0.2 |

| Table 4. Chemical Parameters of SFHP Extracted at Optimized Conditions |
|---------------------------------------------------|
| chemical parameter | SFHP |
| pH | 4.93 ± 0.03 |
| GalA (%) | 82 |

Figure 3: (a) TG/DTG and (b) DSC thermogram analysis of SFHP extracted at optimized conditions.

The SFHP had a pH value of 4.93, whereas high methoxyl pectin extracted from tobacco had a pH of 2.63. Similarly, the obtained SFHP had a DE between 17.7 and 19.4%, which was comparatively lower than other sunflower head pectins extracted by ammonium oxalate and sodium citrate with DE values of 31.7 and 49%, respectively. Note that the SWE process may have decreased the DE. Moreover, the DE from two different methods had a difference of 1.7%. Interestingly, the GalA content was found to be 82%, indicating that the SFHP was of good quality and satisfied the commercial pectin requirement (GalA > 65%). The SFHP was of light yellow color with the required lightness. Lighter colored pectin is a typical commercial product, which is generally preferable for food industry use. The possible reason for the lighter color may be the short heating time compared to the conventional method and raw material without pigmentation.
which can be used to create links and loops between various aromatic clusters. When the pyrolysis temperature increases, these groups are partially destroyed. Consequently, the polyaromatic structures could further stack compactly, and the residual mass was 24.4% at 700 °C.

2.6. Differential Scanning Calorimetry. Figure 3b shows the DSC curve, revealing two peaks: one endothermic peak at 95.6 °C as the melting temperature ($T_m$) and one exothermic peak as the degradation temperature ($T_d$) at 304.5 °C. The endothermic peak was related to DE, $M_w$, and the GalA content of pectin. The $T_m$ value was comparatively lower, which indicated that a lower DE and $M_w$ made pectin absorb water. Therefore, more energy was required to completely remove water because the endothermic phenomenon was attributed to water evaporation. The presence of water might have resulted from hydrogen bonds among entities of GalA and structural transformations from stationary 4C$_1$ chair conformation of the galacturonan ring to the inverse 1C$_5$ chair conformation. Based on this study, it can be said that the SFHP had a strong water retention capacity. The smaller exothermic peak at 225 °C may be caused by impurities in pectin. Both the degradation temperature ($T_d$) and degradation enthalpy ($\Delta H_d$) can reflect the relevant characteristics of thermal cracking. Pectin was pyrolyzed by a random breakdown of glycoside bonds prior to further decomposition. According to the abovementioned results, SFHP had a relatively good thermal stability. Therefore, SFHP could be used as an additive to food products, such as cakes, breads, and pastries, which can be subjected to high temperatures. Thus, high-temperature-resistant pectin could be more favored in the food industry.

2.7. Surface Tension of SFHP. We determined the surface tension of pectin obtained under modified optimal extraction conditions. As shown in Figure 4, the surface tension decreased with the increase in pectin concentration from 0.005 to 1.1% (w/v). After 1.1%, there was little difference in the surface tension values. The lowest surface tension (45.38 mN/m) was obtained from the 1.5% (w/v) aqueous solution of SFHP. In previous studies, the surface tension values of commercial apple pectin solutions, pistachio green hull pectin, and sour orange peel pectin were 63, 49.75, and 42.14 mN/m at a concentration of 0.5% (w/v), respectively. In fact, the enzymatically modified apple pectin had a surface tension value of 55 mN/m because of changes in the blockwise distribution of carboxylic acid groups. Similarly, the surface tension values of sugar beet pulp pectin samples ranged from 48.3 to 58.7 mN/m at a concentration of 0.1% (w/v). Therefore, SFHP reduced the surface tension more efficiently than other reported pectins. The surface activity of pectins may be due to the presence of hydrophobic groups such as methoxy and acetyl groups. Surface tension is one of the most important properties in aerated food products. Note that the surface activity of pectin has a direct relation with its $M_w$. Generally, pectins with lower surface tension values are more appropriate for these products, which may prove to be a promising application of SFHP.

2.8. FTIR Spectrum of SFHP. The FTIR spectrum was used to determine the primary functional groups present in SFHP. As shown in Figure 5A, the strong absorption peak at 3418 cm$^{-1}$ was attributed to the stretching vibration of the O–H groups present in the inter- and intramolecular hydrogen bonding of the GalA backbone. The peak at around 2936 cm$^{-1}$ corresponded to the absorption of the stretching and bending vibrations of the C–H groups (CH, CH$_2$, and CH$_3$) in pectin. The peaks at 1631 and 1741 cm$^{-1}$ were specific to the free carboxylic groups (–COO$^-$) and esterified carboxyl (–COOR), respectively. Similarly, the peak at 1415 cm$^{-1}$ was attributed to C–H stretching vibrations. Moreover, the absorption patterns between 1300 and 800 cm$^{-1}$ were collectively referred to as the “fingerprint” region and reflected about 3418 cm$^{-1}$ was attributed to the stretching vibration of the O–H groups present in the inter- and intramolecular hydrogen bonding of the GalA backbone. The peak at around 2936 cm$^{-1}$ corresponded to the absorption of the stretching and bending vibrations of the C–H groups (CH, CH$_2$, and CH$_3$) in pectin. The peaks at 1631 and 1741 cm$^{-1}$ were specific to the free carboxylic groups (–COO$^-$) and esterified carboxyl (–COOR), respectively. Similarly, the peak at 1415 cm$^{-1}$ was attributed to C–H stretching vibrations. Moreover, the absorption patterns between 1300 and 800 cm$^{-1}$ were collectively referred to as the “fingerprint” region and reflected
specific variations in pectin monosaccharide composition.\textsuperscript{32} Note that absorptions between 1096 and 1012 cm\textsuperscript{-1} were assigned to the R−O−R and C−C rings related to the glycosidic linkage between sugar units in the pectin structure.\textsuperscript{43} The peak at 1010−1100 cm\textsuperscript{-1} showed that there were α-glucoside bonds and β-glicoside bonds. In conclusion, there was an obvious polysaccharide structure in SFHP.\textsuperscript{37}

2.9.\textsuperscript{1}H NMR Spectrum Analysis of SFHP. Figure S8 and Table 5 show the \textsuperscript{1}H NMR spectrum and the fraction of

Table 5. Chemical Shifts of the Glycosyl Residues of the SFHP Fraction from \textsuperscript{1}H NMR Spectra

| glycosyl residues | chemical shifts, δ (ppm) |  
|------------------|--------------------------|
|                  | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | \(-\text{OCH}_3\) |
| →4-\(\alpha\)-Galp-1\(\rightarrow\) | 5.14 | 3.81 | 3.86 | 4.37 | 3.64 | 3.68 |
| →3-\(\alpha\)-Galp-1\(\rightarrow\) | 5.32 | 3.72 | 3.72 | 4.29 | 3.86 | 3.93 |
| →2-\(\alpha\)-Rhap-1\(\rightarrow\) | 4.95 | 4.05 | 3.90 | 3.33 | 3.51 | 1.15 |
| →2,4-\(\alpha\)-Rhap-1\(\rightarrow\) | 4.95 | 4.05 | 3.90 | 3.63 | 3.51 | 1.24 |
| →4-\(\beta\)-Galp-1\(\rightarrow\) | 4.57 | 3.57 | 3.65 | 3.11 | 3.72 | 3.61 |
| →3-\(\beta\)-Galp-1\(\rightarrow\) | 4.55 | 3.57 | 4.04 | 3.95 | 3.63 | 3.61 |
| →6-\(\beta\)-Galp-1\(\rightarrow\) | 4.55 | 3.57 | 3.72 | 3.95 | 3.63 | 4.05 |
| →3,6-\(\beta\)-Galp-1\(\rightarrow\) | 4.55 | 3.57 | 4.04 | 3.95 | 3.63 | 4.05 |
| →3,5,\(\alpha\)-Araf-1\(\rightarrow\) | 5.02 | 4.31 | 4.01 | 4.05 | 3.89 |
| →5,\(\alpha\)-Araf-1\(\rightarrow\) | 5.07 | 4.22 | 3.86 | 4.18 | 3.90 |
| →3,\(\alpha\)-Araf-1\(\rightarrow\) | 4.88 | 3.57 | 3.61 | 3.72 | 3.72 | 3.93 |

SFHP, respectively. A very large and sharp singlet at 3.72 ppm was attributed to the methoxy ester (−\(\text{OCH}_3\)) of GaLA. The signal near 1.98 ppm was attributed to the acetyl groups (−\(\text{COCH}_3\)) of esterified GaLA units,\textsuperscript{49} whereas those at 5.14, 3.81, 3.86, 4.37, and 3.64 ppm were assigned to H-1, H-2, H-3, H-4, and H-5 of GaLA units,\textsuperscript{50} respectively. Similarly, the signal at 4.55 ppm was probably derived from branched groups of β-1,3, β-1,6, and β-1,3,6-linked galactose (GaLA) residues.\textsuperscript{51} The chemical shift of H-1 (5.07, 5.02, and 4.88 ppm) indicated that the \(\alpha\)-1,5, \(\alpha\)-1,3,5, and \(\alpha\)-1,3-linked arabinose (Ara) residues remained.\textsuperscript{52,53} The signals at 1.08 and 1.16 ppm were thought to be indicative of the presence of methyl groups of 1-Rha. Note that the anomeric H-1 signals near 4.95 ppm belonged to \(\alpha\)-1,2 and \(\alpha\)-1,3,4 Rha residues.\textsuperscript{40} Thus, the \textsuperscript{1}H NMR spectrum further confirmed the presence of a pectin polysaccharide structure in the obtained SFHP.

3. CONCLUSIONS

In this study, a green extraction process using subcritical water was applied to fresh sunflower heads for the extraction of pectin. The extraction process was optimized using BBD, resulting in the near-optimal, but practical extraction conditions: pressure of 8 bar, extraction temperature of 120 °C, time of 20 min, and LSR of 7 mL/g with 6.57 ± 0.6% yield of SFHP. Also, the SFHP exhibited low \(M_w\) with a low DE (≤50%), good surface tension lowering properties, and high GaLA content. The SFHP was fully characterized and revealed that fresh sunflower heads were a good source of LMP. This extraction method was free of organic/toxic reagents; thus, this environmentally friendly and efficient use of fresh sunflower heads should contribute toward developing the use of LMP in the food industry.

4. MATERIALS AND METHODS

4.1. Materials and Chemicals. Fresh sunflower heads (moisture content = 550 g/kg) were obtained from a local market in Shanxi, China. Sulfuric acid, sodium sulfate, and ethanol were obtained from Beijing Chemical Works (Beijing, China). Chromatographic grade acetonitrile, potassium bromide, sodium hydroxide, carbazole, and deuterium oxide (\(D_2\)O, 99.9%) were purchased from Aladdin Reagents Co., Ltd. (Shanghai, China).

4.2. Pectin Extraction. We extracted pectin from fresh sunflower heads using a modified method.\textsuperscript{18} Before extraction, leaves, roots, soil, and all other impurities were removed from the fresh sunflower heads. After the heads were manually collected, they were cut into small pieces (1 \(\times\) 1 cm\textsuperscript{-1}), which were washed with water at 100 °C and slowly stirred for 20 min at a solid/water ratio of 1:10. The slurries were filtered through a cheesecloth to remove the soluble pigments and dust.\textsuperscript{36} The extraction conditions were optimized by single-factor experiments. To identify and set the range of extraction variables for optimizing the extraction process using the BBD, several extractions were performed by changing one parameter at a time while setting the others constant. The experimental variables were extraction time (10−50 min), LSR (2−8 mL/g), temperature (100−140 °C), and pressure (2−10 bar). Immediately after the SWE reaction, the extract was obtained by filtration using a 400-mesh gauze. After rotary evaporation, twice the volume of 95% (v/v) ethanol was added. Precipitation of jellified pectin from ethanol was allowed to occur for 24 h at 25 °C. Finally, the jellified pectin was filtered using a 300-mesh gauze, and the solid was dried to obtain the pectin yield percentage \(Y\) (%) using the following formula

\[
Y\ (%) = \frac{m_0}{m} \times 100 \tag{2}
\]

where \(m_0\) (g) is the mass of dried pectin and \(m\) (g) is the mass of fresh sunflower heads.

4.3. Galacturonic Acid (GaLA) Content. Because GaLA was the dominant monosaccharide in the pectin structure, its content was determined as the indicator of pectin yield by the colorimetric carbazole method.\textsuperscript{18} We added 6 mL of \(H_2\)SO\(_4\) (98% w/w) to 1 mL of the sample (50 mg/L), and then the mixture was agitated with a vortex mixer. The tubes were cooled in an ice−water bath and heated in boiling water for 15 min and then immediately cooled in an ice bath. After cooling to room temperature, 0.5 mL (1.5% w/v) of carbazole−ethanol was added. The tubes were shaken and the reaction was allowed to stand at room temperature for 30 min. The absorbance reading at 530 nm was obtained using a UV−vis spectrophotometer (PerkinElmer). The absorbance values were compared using the GaLA calibration curve. The GaLA yield can be obtained as follows

\[
\text{GalA} \ (%) = \frac{0.2 \times c}{10} \times 100 \tag{3}
\]

where \(c\) (mg/L) is the GaLA content calculated by the calibration curve.
4.4. Determination of Pectin pH. SFHP (1.25 g) was dissolved in distilled water (50 mL) and the pH was measured at 25 °C using a pH meter.\(^{41}\)

4.5. Determination of Pectin Esterification. 4.5.1. FTIR Method. SFHP was analyzed using a Bruker Tensor 27 Fourier transform infrared spectrometer (Bruker, Germany). The dried sample (1 mg) and potassium bromide (100 mg) were ground together and pressed into pellets. The pellet was then scanned 32 times over 4000–400 cm\(^{-1}\) with a resolution of 2 cm\(^{-1}\), and the resultant spectra were smoothed to remove noise. Following the method of Pappas et al.,\(^{55}\) the pectin samples were prepared and a calibration curve was established. The DE of SFHP was determined according to the calibration curve of dextran standards (2.8, 20.4, 62.9, 111.9, 212.5, 310.2, and 390 kDa).

4.5.2. Titrimetry Method. The DE of SFHP was determined by titration using a slightly modified method.\(^{43}\) Briefly, a dried sample (200 mg) was moistened with absolute ethanol (1 mL) and then dissolved in distilled water free of carbon dioxide (20 mL) at 40 °C. After dissolving the sample completely, five drops of phenolphthalein reagent were added, and then the sample was titrated with 0.1 mol/L NaOH (\(V_1\), mL). Then, 0.5 mol/L NaOH (20 mL) was added and the sample was shaken for 4 h to encourage a hydrolysis reaction. Next, 0.5 mol/L HCl (20 mL) was added and the sample was shaken until the pink color disappeared. Finally, five drops of phenolphthalein reagent were added and the mixture was titrated with 0.5 mol/L NaOH until a pale pink color persisted even after vigorous shaking (\(V_2\), mL). DE was computed using the following equation

\[
DE = \frac{V_1}{(V_1 + V_2)} \times 100
\]

4.6. Molecular Weight Determination. We determined the \(M_w\) of the extracted pectin using high-performance size exclusion chromatography (HPSEC) coupled with a Shodex GF-7M HQ (50 \(\mu\)m, 7.8 \(\times\) 300 mm) and a refractive index detector (RID) 201H at 35 °C. The extract was dissolved in a NaSO\(_4\) solution (0.25 mg/mL) and passed through a 0.22 \(\mu\)m membrane filter. This was followed by manually injecting the extract through a 50 \(\mu\)L loop. The mobile phase was a 0.05 mol/L NaSO\(_4\) solution with a flow rate of 0.5 mL/min. The \(M_w\) was determined according to the calibration curve of dextran standards (2.8, 20.4, 62.9, 111.9, 212.5, 310.2, and 390 kDa).

4.7. Thermogravimetric Analysis. The thermal stability test was performed to evaluate the thermal degradation of SFHP. A Mettler Toledo TGA-1 thermogravimetric analyzer (Mettler Toledo, Switzerland) with flowing nitrogen (50 mL/min) was used to determine the thermal stability of SFHP (5 mg) in the temperature range of 25–700 °C at a rate of 10 °C/min. Within the heating range, the weight loss rates because of thermal decomposition were calculated by comparing the original weight with data of weight loss.

4.8. Differential Scanning Calorimetry. A Mettler Toledo DSC1 instrument was used to determine the thermal analysis of SFHP. The sample (5 mg) was added into a sealed aluminum pan with a pinhole alongside an empty pan used as a reference.

The temperature was heated from 25 to 350 °C at a rate of 10 °C/min. Nitrogen was the conveying gas employed at a flow rate of 20 mL/min.

4.9. Surface Tension. Surface tension values were measured using a Kruss-K100 tensiometer (Kruss, Germany) with a platinum ring (precision range ±0.5 mN/m). Samples (0.005–1.5% w/v) were prepared in distilled water, and the surface tension measurements were performed at 25 °C. A surface tension value of 72.5 mN/m was obtained for distilled water and was used as a reference. The platinum ring was washed with distilled water, flame-dried over an alcohol lamp, and cooled to room temperature prior to use.

4.10. Nuclear Magnetic Resonance Spectra. SFHP obtained under optimal conditions was dehydrated and dissolved in D\(_2\)O (0.55 mL, 99.9%). Then, \(^1\)H NMR analysis was performed at 20 °C using a Varian Unity Bruker 600 MHz spectrometer (Bruker, Germany). The data were collected and analyzed using the MestReNova software.

4.11. Experimental Design. The type of the experimental design chosen is BBD, while RSM is the mathematical method used to analyze the results of the experimental design (i.e., finding the optimal conditions). The parameters ranges were fixed based on our preliminary experiments as follows: extraction pressure (6–10 bar), temperature (100–140 °C) and LSR (4–8 mL/g). However, a BBD with three variables in three levels was applied for evaluating the effect of the different factors on the extraction yield. To validate the theoretical accounts for the optimization process, an ANOVA was performed.

The optimal conditions were estimated using a second-order polynomial equation, and then three-dimensional response surface plots were drawn between each independent and dependent variables. The generalized form of the second-order polynomial equation is as follows

\[
Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_i^2 X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j
\]

where \(Y\) is the response; \(X_i\) and \(X_j\) are variables; \(\beta_0\) is the model intercept coefficient; and \(\beta_i, \beta_i^2\) and \(\beta_{ij}\) are the interaction coefficients of the linear, quadratic, and the second-order terms, respectively. All computations and graphics in this study were conducted using the statistical software Design Expert 7.0 and Origin 8.0.

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