Green and Efficient Extraction Approach for Polyphenol Recovery from Lotus Seedpods (Receptaculum Nelumbinis): Gas-Assisted Combined with Glycerol

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ABSTRACT: In this paper, the gas-assisted combined with glycerol extraction (GAGE) for polyphenol recovery from lotus seedpods (LSPs) was modeled and optimized. Box−Behnken design was applied to optimize the total polyphenol content (TPC) of LSP along with enhancing antioxidant activities using response surface methodology based on the TPC extraction yield (%), which was affected by glycerol concentration, time, temperature, and glycerol-to-solid ratio. The optimal conditions for the LSP extract were glycerol-to-solid ratio, 42 mL/g; time, 50 min; concentration of glycerol, 45%; and temperature, 70 °C. Ultra-high-pressure liquid chromatography integrated with triple-time-of-flight mass spectrophotometry (UPLC-Triple-TOF/MS) analysis revealed nine biologically active polyphenols. Furthermore, Fourier-transform infrared spectroscopy and scanning electron microscopy results demonstrated the effect and influence during extraction. The findings suggested that GAGE is a potential, green, and high-efficiency alternative that could be used to recover polyphenols from plant source byproducts.

1. INTRODUCTION

As ornamental aquatic plant, lotus (Nelumbo nucifera Gaertn.), is cultured and distributed throughout Asia, including China.1−3 Lotus is an edible plant, and all its parts contain natural compounds with medicinal uses in Chinese traditional medicine.4−6 Lotus seedpods (LSPs), the inedible parts of the lotus, are discarded byproducts of a lotus during processing. However, due to the fact that LSPs are rich in gallic acid, catechin, and other polyphenols,7−9 which have many good biological efficacies, including antimicrobial, antioxidant, and antitumor activities and so on,8,10 they have attracted extensive attention in Oriental medicine or health care industries. Thus, getting more polyphenols is crucial for LSP. However, traditional extraction methodologies for polyphenols like Soxhlet and macerative extraction give a low efficiency and a low yield. Hence, the development of new advanced alternatives is expected to overcome the drawbacks, including microwave-assisted, ultrasonic-assisted, subcritical fluid, supercritical fluid, gas-assisted, and so on.9,11−15 Among these, gas-assisted extraction is an uncomplicated, rapid, and efficient alternative to the traditional extraction method and is especially environmentally friendly.

As for extraction solvents, for instance, methyl alcohol and other conventional organic solvents16,17 which were used widely due to their dissolution ability and extraction efficiency, faced the challenges of being the potentially environmentally hazardous and unacceptable solvent residues in the extracts.18 Meanwhile, although ethyl alcohol is known to be a GRAS (generally recognized as safe) solvent, it also faced the major limitation of the residue removal cost. Therefore, green solvents considered environmentally friendly were required alternatives to the harmful organic solvents. Glycerol is a valuable green solvent that has so many advantages, that is, it is a non-toxic, natural, and inexpensive viscous bioliquid and dispensed with any post extraction steps. Glycerol can also change the polarity of water. Thus, it can be used to increase the recovery of polyphenols from various plant tissues.19,20

Current investigation is sought to develop a modern technique based on gas-assisted combined with glycerol extraction (GAGE) from LSP, which is more efficient and effective for extracting polyphenols known for their high biological activities. In addition, the technique can overcome the shortcomings of traditional extraction techniques based on hazardous organic solvents that have a negative impact on the environment or the cost limitation of removing the residues after extraction.
2. RESULTS AND DISCUSSION

2.1. Model Fitting. Single-factor experiments were conducted to study the influence of total polyphenol content (TPC) extracted from LSP. According to the outcome, as shown in Figure 1A–D, the independent factor ranges were chosen. The experimental design matrix for TPC extraction yield from LSP is shown in Table 1. Due to the effect of gas flow on TPC extraction, the yield was not significant ($p > 0.05$), so it was fixed to 0.75 L/min. After that, Box–Behnken design (BBD) comprised 4 process parameters, 3 coded levels were used, and 29 experiments were conducted (Table 2) to optimize the GAGE process and maximize TPC extraction yield. Meanwhile, response surface regression analysis was performed, and the two-order polynomial equation was derived according to the obtained data shown in eq 1

\[
TPC = +89.18 + 1.02X_1 + 0.14X_2 - 0.43X_3 + 5.65X_4 \\
- 0.92X_1X_3 + 0.61X_1X_4 + 0.16X_2X_3 + 0.73X_2X_4 \\
- 0.67X_2X_4 - 0.10X_2X_4 - 4.15X_2^2 - 2.63X_2^2 \\
- 6.42X_3^2 - 1.6X_4^2 
\]  

(1)

Experimental data were analyzed through ANOVA (Table 3) to investigate the proposed model, and the result revealed that the model response was remarkably significant ($p < 0.001$). At the same time, the lack of fit was not significant. The $p$-value of 0.5230 conferred that the model term can determine the model’s adequacy and can describe the relationship between the observed data and model response. Meanwhile, the $F$-value of the model, $R^2$, and adjusted $R^2$ were 378.86, 0.9974, and 0.9947, respectively, which demonstrated that the model displayed high positive correlation between experimental and predicted values and could be used to predict the values.

For the response of TPC, the result of ANOVA shown in Table 3 revealed that the linear parameters, that is, glycerol-to-solid ratio ($X_1$) and temperature ($X_4$), exhibited a remarkably significant effect, and the quadratic parameters, such as $X_1^2$, $X_3^2$, and $X_4^2$, also displayed a remarkably significant effect. In addition, the interactions, $X_1X_2$, $X_1X_3$, $X_2X_3$, and $X_3X_4$, exhibited significant ($p < 0.01$) effects on TPC. According to the result of the second-order polynomial, the linear effects of glycerol-to-solid ratio ($X_1$), time ($X_2$), and temperature ($X_4$) showed positive effects on TPC extraction yield. The interaction effects, such as $X_1X_2$, $X_1X_3$, $X_2X_3$, and $X_3X_4$, also illustrated a positive TPC extraction yield. All the quadratic effects yielded remarkably significant negative effects on TPC. Similarly, the interaction effects $X_1X_2$ and $X_3X_4$ showed negative effects on TPC.

Figure 2 illustrates the interaction of independent factors and their effects on TPC extraction yield, which were realized by drawing two independent variables on the reaction while maintaining the other two variables at zero levels. In terms of interaction effects, glycerol-to-solid ratio and time with significant adverse effects on TPC (Figure 2A) indicated that TPC decreases with increasing glycerol-to-solid ratio and extraction time. According to Figure 2A, the glycerol-to-solid ratio in medium values resulted in the best TPC extraction yield. This is because mass transfer becomes more efficient with increasing solution volume. However, with the continual increase of glycerol-to-solid ratio, the mass-transfer effect

![Figure 1. Influence of (A) glycerol-to-solid ratio, (B) time, (C) concentration of glycerol, and (D) temperature on TPC; (E) kinetic curve of time extraction. Data are expressed as the mean ± SD (n = 3). Letters a, b, c, d, e, f, and g revealed a significant difference ($p < 0.05$).](https://doi.org/10.1021/acsomega.1c04190)
decreased. Obviously, the gas agitation cannot make enough contact between a solution with LSP; therefore, it reduced the concentration gradient and mass transfer, which led to the reduced TPC extraction efficiency.

Similarly, time in medium values also resulted in the best yield due to the fact that the prolonged extraction time can promote the total solubility of TPC. In contrast, the yield of TPC decreased by continuing to prolong the extraction time, which was consistent with the previous study of Marti-García et al. Such an effect might occur due to exposure to bioactive compounds, especially the thermal degradation of TPC. Figure 2B displays the interaction between glycerol-to-solid ratio and concentration of glycerol with a significant positive effect on TPC. The concentration of glycerol is a crucial factor for the yield of TPC as increasing glycerol concentration can enhance the yield of TPC because it might enhance soluble polyphenols extracted from LSP or might change the dielectric constant and make the polarity of water reduce, thus extracting polyphenols more effectively. In the meantime, with the increasing glycerol concentration, glycerol changed the solvent’s polarity and enhanced the extraction of bioactive substances. Different solvents have different solubilities of polyphenols. Additionally, it is a very complicated phenomenon based on not only the mechanism of their polarity, which might explain the solvent extraction efficiency, but also the intermolecular forces between the plant matrix and the solvents. Furthermore, the external influences which come from the gas also affected the extraction efficiency of glycerol for LSP. Besides this, TPC extraction yield enhanced with the increase of glycerol-to-solid ratio because of the solution volumes. Figure 2C shows the interaction between time and concentration of glycerol with a significant positive influence on TPC extraction. A prolonged extraction time and

| Table 2. BBD for TPC with 29 Experimental and Predicted Values |
|---------------------------------------------------------------|
| **runs** | glycerol-to-solid ratio (mL/g) | time (min) | concentration of glycerol (%) | temperature (°C) | experimental values (mg GAE/g) | predicted values (mg GAE/g) |
|----------|-------------------------------|-----------|-------------------------------|----------------|-------------------------------|----------------------------|
| 1        | 30.00                         | 50.00     | 45.00                         | 40.00          | 76.68                         | 76.92                      |
| 2        | 30.00                         | 50.00     | 35.00                         | 55.00          | 78.45                         | 78.62                      |
| 3        | 40.00                         | 50.00     | 45.00                         | 55.00          | 89.40                         | 89.18                      |
| 4        | 50.00                         | 40.00     | 45.00                         | 55.00          | 84.09                         | 84.21                      |
| 5        | 30.00                         | 40.00     | 45.00                         | 55.00          | 80.57                         | 80.31                      |
| 6        | 40.00                         | 50.00     | 55.00                         | 70.00          | 86.35                         | 86.48                      |
| 7        | 40.00                         | 60.00     | 45.00                         | 40.00          | 80.17                         | 80.11                      |
| 8        | 40.00                         | 60.00     | 55.00                         | 55.00          | 80.24                         | 80.56                      |
| 9        | 30.00                         | 60.00     | 45.00                         | 55.00          | 82.68                         | 82.44                      |
| 10       | 50.00                         | 50.00     | 45.00                         | 70.00          | 90.49                         | 90.27                      |
| 11       | 40.00                         | 50.00     | 35.00                         | 70.00          | 86.76                         | 87.15                      |
| 12       | 50.00                         | 50.00     | 45.00                         | 40.00          | 78.45                         | 78.64                      |
| 13       | 50.00                         | 60.00     | 45.00                         | 55.00          | 82.50                         | 82.64                      |
| 14       | 40.00                         | 50.00     | 45.00                         | 55.00          | 89.58                         | 89.18                      |
| 15       | 40.00                         | 60.00     | 35.00                         | 40.00          | 82.50                         | 82.64                      |
| 16       | 50.00                         | 50.00     | 45.00                         | 55.00          | 79.89                         | 79.81                      |
| 17       | 30.00                         | 50.00     | 55.00                         | 55.00          | 76.29                         | 76.05                      |
| 18       | 40.00                         | 50.00     | 55.00                         | 40.00          | 75.49                         | 74.98                      |
| 19       | 50.00                         | 50.00     | 35.00                         | 55.00          | 79.62                         | 79.46                      |
| 20       | 40.00                         | 40.00     | 45.00                         | 40.00          | 78.11                         | 78.50                      |
| 21       | 40.00                         | 60.00     | 45.00                         | 70.00          | 90.37                         | 90.08                      |
| 22       | 40.00                         | 50.00     | 45.00                         | 55.00          | 88.81                         | 89.18                      |
| 23       | 40.00                         | 50.00     | 45.00                         | 55.00          | 88.78                         | 89.18                      |
| 24       | 40.00                         | 50.00     | 35.00                         | 55.00          | 79.83                         | 79.97                      |
| 25       | 40.00                         | 40.00     | 55.00                         | 55.00          | 78.94                         | 78.83                      |
| 26       | 40.00                         | 40.00     | 45.00                         | 70.00          | 90.99                         | 91.14                      |
| 27       | 30.00                         | 50.00     | 45.00                         | 70.00          | 88.07                         | 86.98                      |
| 28       | 40.00                         | 50.00     | 45.00                         | 55.00          | 89.36                         | 89.18                      |
| 29       | 40.00                         | 40.00     | 35.00                         | 55.00          | 81.44                         | 81.15                      |

| Table 3. ANOVA of the Fitting Quadratic Polynomial Model in the Response Surface |
|-----------------------------------------------|
| **source** | sum of squares | mean square | F-value | p-value | Prob > F |
|----------|---------------|-------------|---------|---------|----------|
| model    | 739.70        | 52.84       | 378.86  | <0.0001 |          |
| Linear   |               |             |         |         |          |
| X1: glycerol-to-solid ratio | 12.60 | 12.60 | 90.32 | <0.0001 |          |
| X2: time | 0.23          | 0.23        | 1.65    | 0.2197  |          |
| X3: concentration of glycerol | 2.25 | 2.25 | 16.15 | 0.0013  |          |
| X4: temperature | 383.31 | 383.31 | 2748.50 | <0.0001 |          |
| Interaction | 3.42 | 3.42 | 24.51 | 0.0002  |          |
| X1X2 | 1.48          | 1.48        | 10.59   | 0.0058  |          |
| X1X3 | 0.11          | 0.11        | 0.77    | 0.3962  |          |
| X1X4 | 2.13          | 2.13        | 15.25   | 0.0016  |          |
| X2X3 | 1.80          | 1.80        | 12.90   | 0.0030  |          |
| X2X4 | 0.040         | 0.040       | 0.28    | 0.6023  |          |
| X3X4 | 0.840         | 0.840       | 0.28    | 0.6023  |          |
| Quadratic | 111.90 | 111.90 | 802.35 | <0.0001 |          |
| X12 | 44.95          | 44.95       | 322.28  | <0.0001 |          |
| X22 | 267.73        | 267.73      | 1919.75 | <0.0001 |          |
| X32 | 16.52          | 16.52       | 114.83  | <0.0001 |          |
| lack of fit | 1.42 | 0.14 | 1.06 | 0.5230 |          |
an increased glycerol concentration can promote the mass transfer, resulting in the best TPC extraction yield when these are in the medium values. Figure 2D reveals that the interaction between time and temperature significantly adversely affected TPC. Temperature is also an important factor for TPC extraction yield. An increase in temperature at a certain threshold initiates the mass transfer, facilitating the TPC diffusion from LSP under heat activation, which led to increased extraction. The sample becomes soft because of heating, which weakened the interaction of polyphenols and made more polyphenols diffuse into the solvent. In reality, with temperature within a specific range, the yield of TPC may be affected only by thermal instability. Once the temperature was exceeded, the extraction yield would decrease. This is likely due to the fact that the high temperature led to the oxidation and degradation of TPC, which affects the extraction system.

Additionally, the TPC extraction yield was improved through response surface methodology (RSM) significantly, without neglecting the interaction of independent variables. The optimal conditions obtained by RSM for LSP were glycerol-to-solid ratio, 41.56 mL/g; time, 48.68 min; concentration of glycerol, 44.74%; and temperature, 70 °C.

Based on the result of TPC with optimal conditions obtained by RSM, the kinetic conditions for extraction were adjusted to the nearest integer numbers, that is, a glycerol-to-solid ratio of 42 mL/g, a time of 50 min, a concentration of glycerol of 45%, and a temperature of 70 °C.

2.2. Kinetics of Extraction. Figure 1E shows the kinetic behavior of TPC extraction yield, which was affected by time. Kinetic extraction was evaluated up to 50 min according to the result of RSM since a long time with a high temperature could be prejudicial to polyphenols. The extraction rate of TPC increased with the extension of time. TPC extraction yield increased with prolonging time from 0 to 50 min, which indicated that the LSP diffused to glycerol solution. Glycerol solution penetrates solid particles, and phenols rapidly spread into the solvent. Meanwhile, diffusion phenomena can be divided into three phases: fast with non-rate-limiting, slow with rate-limiting, and non-rate-limiting. During the first phase, glycerol solution penetrated the solid particles, which led the polyphenols to rapidly diffuse to the solvent. Then, the second-phase diffusion was slowed down, which might be due to polyphenol diffusion from the inner particle to the solid–liquid interface, which was called “regular regime” according to Rakotondramasy-Rabesiaka because the extraction rate will increase slightly for a long time. Finally, in the third phase, with the increase of time, the TPC extraction yield still increased because of the diffusion from the solid–liquid interface into glycerol solution.

2.3. Model Validation. This step aimed to verify the model validity; hence, a further experiment was enforced under the optimum extraction conditions, the same as the extraction conditions obtained by RSM.
kinetic conditions. The investigation was conducted in triplicate, and the mean value was recorded. The result of TPC was 93.27 ± 3.29 mg GAE/g LSP, and the predicted one was 93.37 mg GAE/g LSP, with a confidence interval of 0.9947, an error coefficient of 0.11%, and a variation coefficient of 0.45%. Here, there was no significant difference between the indicated and experimental values. Therefore, the model was considered accurate and reliable and could be used to show the extraction process of TPC from LSP.

2.4. Basal Content Determination of Total Flavonoid Content, Total Condensed Tannin Content, and Total Tannin Content. As shown in Table 4, total flavonoid content (TFC), total condensed tannin content (TCTC), and total tannin content (TTC) were determined to compare the content of TPC extraction yield in the extraction process, which was considered accurate and reliable and could be used to show the extraction process of TPC from LSP.

| sample | TFC (mg QE/g) | TCTC (mg CE/g) | TTC (mg TAE/g) |
|--------|---------------|----------------|----------------|
| GAGE   | 45.98 ± 2.03b | 72.00 ± 2.39a  | 111.58 ± 1.21a |
| WG     | 74.46 ± 3.46a | 54.24 ± 0.48b  | 73.27 ± 1.03b  |

“Data are expressed as the mean ± SD (n = 6); letters a and b reveal the significant difference (p < 0.05).

The obtained results showed that GAGE had a significantly higher content of TCTC (72.00 ± 2.39 mg CE/g) and TTC (111.58 ± 1.21 mg TAE/g) compared with WG (54.24 ± 0.48 mg CE/g and 73.27 ± 1.03 mg TAE/g, respectively), while WG was significantly richer in TFC (74.46 ± 3.46 mg QE/g) than GAGE (45.98 ± 2.03 mg QE/g). The results implied that TTC had the highest quantities of TPC in GAGE and WG. Thus, a high temperature was a vital factor for achieving a high TPC extraction yield in the extraction process, which was correlated with the previously published research. Moreover, glycerol modified the polarity of the medium and changed the dielectric constant of water, thus improving the solubility of polar polyphenols. Besides, the intermolecular forces between polyphenols and the solvent also affected the solubility and diffusion of the TPC.

Furthermore, the gas-assisted process can enhance the mass transfer because the gas can increase the tube’s pressure, or agitation produced by the gas can also increase the mass transfer. Therefore, TCTC and TTC with GAGE treatment showed a higher content than WG. Still, TFC with GAGE treatment showed a lower content than WG, which may be due to increased solubility of TCTC and TTC in the solution, or a high temperature with the gas caused the degradation of TFC or LSP attachment to the tube by the agitation of the gas.

2.5. Antioxidant Activities. Antioxidant activity analysis assessed the potential of extracts in preventing oxidative stress damage. It depended on the efficiency of antioxidant detection. Polyphenols are easy to be oxidized in the environment because of having a unique aromatic smell and weak acidity, which also relied on the structure of the compounds (the number of the aromatic rings and the hydroxyl groups). Therefore, chelating Fe²⁺ can prevent oxidative damage and hydroxyl free radicals from effortlessly passing through the cell membrane and protect tissues and cells from damage. Thus, the antioxidant activities of LSP under optimized conditions with GAGE and WG treatments were studied by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2, 2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and reducing activity (RA), the four in vitro antioxidant assays. DPPH radical scavenging capability of the extract decided the DPPH assay, and ABTS analysis was similar to the DPPH assay, which reacted by capturing free radicals. The FRAP assay was applied to determine the ability to reduce Fe³⁺ to Fe²⁺, and the RA assay depends on its reduction by giving electrons and scavenging free radicals. Figure 3 shows the improvement of the extraction of polyphenols by GAGE compared with WG on antioxidant activities. GAGE was significantly higher than WG on all the four different antioxidant assays. This is due to the fact that GAGE may reduce the surface tension of glycerol and increase the diffusion and solubility rate and mass transfer of the analyses, enabling the development of polyphenols. As mentioned before, concerning TFC, WG was significantly higher than GAGE. However, GAGE was significantly higher than WG on antioxidant assays, which indicated that the antioxidant activities resulted in the subgroups of polyphenols. The phenolic compounds extracted under premium conditions that might contribute to the antioxidant activity were analyzed using ultra-high-pressure liquid chromatography integrated with triple-time-of-flight mass spectrophotometry (UPLC-triple-TOF/MS).

Nine polyphenols (procyanidin B1, procyanidin B2, catechin, myricetin 3-glucuronide, hyperoside, quercetin 3-O-glucuronide, kaempferol 3-glucuronide, isorhamnetin 3-glucoside, and quercetin) of LSP extract were identified and are shown in Figure S10, which were the base peak chromatograms by comparison with the data from reference compounds in the previous studies. Among these compounds, Quercetin 3-O-glucuronide was the main flavonoid found in the extracts, corroborating with the previous study of Huang.

Figure 3. Antioxidant activities: (1) DPH, (2) FRAP, (3) ABTS, and (4) RA of TPC with GAGE and WG treatments. Note: Data are expressed as the mean ± SD (n = 5); letters a and b reveal the significant difference (p < 0.05).
Quercetin is an abundant naturally occurring flavonoid observed in fruits and vegetables, which was the main compound on the antioxidant activity according to Rivai because of the reactivity with phenol, providing hydrogen atoms.

2.6. FTIR Analysis. Fourier-transform infrared (FTIR) spectroscopy was performed to distinguish and clarify the functional groups that could indicate polyphenols in the LSP extracts obtained from two treatments of GAGE and WG under the maximum conditions. The typical appearances of absorption spectra are illustrated in Figure 4.

Many peaks were identified in the extract with a wavenumber range of 3382–857 cm⁻¹, which were distinguished according to the reference standards and the previous work related to the specific molecular groups. Generally, the absorption band at wavenumbers between 3300 and 3500 cm⁻¹ was allocated to the stretching vibration or O–H wagging, which may be due to polyphenols in the LSP. Thus, the absorption band at 3382 and 3370 cm⁻¹ might result in polyphenols. The absorption band in the scope of 2800–3000 cm⁻¹ belonged to the C–H stretch vibration, which could indicate the presence of methylene compounds or lipids. Therefore, the absorbances at 2930, 2920, 2881, and 2880 cm⁻¹ were associated with C–H stretching in LSP extracts and could be due to glycerol’s presence. The peaks at 2450, 2440, 2320, and 2310 cm⁻¹ were unidentified spectra. Infrequent spectra at 1600–1850 cm⁻¹ indicated the carbonyl groups (C=O), and the peaks at 1650 cm⁻¹ were allocated to the presence of alkenes (C=C), which could indicate the presence of polyphenols and flavonoids. Similarly, the peaks at 1380 and 1370 cm⁻¹ were allocated to bent carboxylic acid, which could indicate the presence of tannins and flavonoids.

Figure 4. FTIR analysis of LSP extracts with GAGE and WG.

2.7. SEM Analysis. To better understand how the GAGE treatment indicated more TPC extraction yield, the microstructures of LSP under GAGE and WG were observed using scanning electron microscopy (SEM) with 300 magnifications and a 2 kV accelerating voltage, and Figure 5 shows the SEM images, from which the outermost layers of LSP were intact and smooth before extraction. During extraction, it can be observed in the figure that an increasing number of leaf structures were uncovered. In the cases of glycerol, the outermost layers were slightly damaged and displayed wrinkles irregularly. Moreover, it appeared to have a rougher surface, and some round particles were exposed on the surface of LSP, which was in agreement with the previous study. When treated with GAGE, the samples’ surface was significantly damaged, and the round particles almost disappeared. This may explain why GAGE treatment is better than WG, resulting in more release and diffusion of TPC extracted from LSP to the solvent.

3. MATERIALS AND APPROACHES

3.1. Plant Materials and Reagents. LSPs (in the old stage) were sourced in Hangzhou in September 2019 and were stored away from light at 4 °C. All solvents were of analytical reagent grade and bought from Aladdin Reagent Co., Ltd. (Shanghai, China).

3.2. Preparation of the Sample Extract. LSP was dried at 45 °C using a hot air oven until constant weight and then...
ground with a grinder (Huangcheng, HC-280T, China) into 40 meshes and subsequently was stored at −20 °C until the extraction. GAGE, as shown in Figure S1, was used to extract the phenol compounds from LSP. Dried and powdered samples (0.5 g) were added into a plastic centrifuge tube (50 mL) and extracted with the required different glycerol concentrations and volumes at different temperatures for different times. Then, the sample was centrifuged for 15 min at 10,950g (Bioridge, TGL-16M, China), and the supernatants were collected. Meanwhile, the WG method was carried out to extract polyphenols and compare with the GAGE method under the optimal GAGE conditions for LSP.

3.3. Experimental Design. BBD with four variables, that is, glycerol-to-solid ratio (X1), time (X2), the concentration of glycerol (X3), and temperature (X4), has been chosen to obtain the optimum parameters on the responses. Tables 1 and 2 show the code levels and the 29 experimental runs. The second-order polynomial (eq 2) can well coincide with the experimental data, which RSM obtained. Here, Yi, k0, k1, k2, k3, k4, and X1, X2, X3, and X4 denote the dependent variable, the regression coefficients, and the process variables, respectively. Design expert software (8.0.6) was applied to build the model and analyzed results. Analysis of variance (ANOVA) was performed for each response by comparing obtained experimental values with the predicted values with a 95% confidence level. Subsequently, the response surfaces of all variables decided the optimal conditions of GAGE from LSP.

\[
Y = k_0 + \sum_{i=1}^{4} k_iX_i + \sum_{i=1}^{4} k_{ii}X_i^2 + \sum_{i=1}^{4} \sum_{j=2}^{4} k_{ij}X_iX_j
\] (2)

3.4. Extraction Kinetics. The optimal conditions of GAGE from LSP were obtained using BBD, by which the time of extraction kinetics was chosen to be 50 min, and it was carried out by collecting samples every 2 min during the first 10 min and every 5 min from 10 to 50 min. Each extraction phase was performed three times, and all the collected samples were measured to determine the polyphenols.

3.5. Analysis of Polyphenols. The TPC of all samples was taken from the previous work of Limwachiranon et al.,52 with certain modifications, and the results are represented as mg gallic acid equivalent (GAE) per gram of LSP (mg GAE/g), which was calculated based on the calibration curve.

The determination of TFC, TCTC, and TTC was done by using the previous approach, according to Bao et al.26

3.6. Analysis of Antioxidant Activities. According to the approach described by Duan et al.53 the DPPH ability of the LSP extract was analyzed with some adjustments. Also, µmol ascorbic acid equivalent (AAE) per g LSP (µmol AAE/g) was used to express DPPH ability.

According to the approach used by Oldoni et al.54 the FRAP activity of the LSP extract was tested, and µmol Fe**/g was used to express the results.

The ABTS+ ability of the LSP extract was analyzed according to Marmouzi et al.55

The RA ability of the LSP extract was analyzed by using the previous work reported by Oyau.56

3.7. UPLC-Triple-TOF/MS Analysis. Waters UPLC (Waters Corp., USA) integrated with a Triple-TOF System (AB SCIEX, USA) was used to analyze the GAGE extractions from LSP under optimum conditions based on Huang et al.40 with a slight modification. First, a sample of 5 µL was injected into an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm; Waters Corp., USA) with a temperature of 40 °C, and the gradient elution (the mobile phases of A and B were 0.1% formic acid–water and 0.1% formic acid-acetonitrile, respectively) with a flow rate of 0.5 mL/min was applied according to the following profile: 0/5, 10/50, 15/95 (min/B %); then, the results were monitored at 254 nm by using a UV detector. The optimum MS conditions in the negative ion mode are as follows: the source voltage and the temperature were −4.5 kV and 550 °C, respectively; the scan ranges of m/z of the precursor ion and product ion were set as 100–2000 and 50–2000 Da, respectively; and the collision energy was set at 10 V.

3.8. FTIR Spectrometry Analysis. The dried LSP extract samples with two different methods were mixed with potassium bromide ground into powder and pressed into a thin sheet. An FTIR spectrometer (Thermo Scientific, Nicolet iS50 Spectrometer, USA) was used to conduct the infrared analysis at room temperature, which was fitted with OMNIC software. The range of wavenumbers and the scans of the FTIR spectra were 4000–400 cm⁻¹ and 32, respectively. Meanwhile, the resolution rate was chosen to be 4 cm⁻¹. The air spectrum was used as a background spectrum to correct the spectrum by using single spectra. EXCEL was used to do statistical calculations.

3.9. Scanning Electron Microscopy. The Microscopic analysis of the dried LSP (before and after extraction treatments) samples was conducted according to the approach of Zhou et al.57 A Gemini SEM 300 (Carl Zeiss, Germany) was used to visualize the surface morphology of the prepared samples using a double-sided adhesive carbon tape to install the samples on aluminum stubs and spraying gold–palladium on the surface of the powder.

3.10. Statistical Analysis. Ibmspp20 software was used to analyze the data, and IBM SPSS 20 software was performed to analyze the data. One-way ANOVA and the Tukey test were used for statistical analysis of significance of the model, and 0.05 was considered as a significance level. All treatments were done three times, and the mean ± standard deviation (SD) was used to express the results.

4. CONCLUSIONS

In this study, LSP polyphenol extraction was investigated using GAGE, which was demonstrated to be a green and high-efficiency approach to replace the conventional extraction. With BBD and kinetic methodologies, the effect of variables on GAGE and the optimum process were studied. Moreover, the mathematical model with high correlation provided the effectiveness of the optimized conditions of GAGE on polyphenol extraction. The antioxidant activities could be ascribed to the identified nine polyphenols, demonstrated using the in vitro antioxidant assay, that is, DPPH, ABTS, FRAP, and RA. GAGE’s beneficial effect can be used as a green and high-efficiency alternative approach to extract polyphenols from argo-products; additionally, the process can be environmentally friendly.

ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04190.

Equipment for gas-assisted extraction; standard curve of gallic acid for TPC; standard curve of Quercetin for
TFC; standard curve of catechin for TCTC; standard curve of tannin acid for TTA; standard curve of ascorbic acid for DPPH; standard curve of Fe^{2+} for FRAP; standard curve of Trolox for ABTS; standard curve of Trollox for RA; basic peak chromatograms of Receptor- 
ulmum nelumbinis extract obtained by the gas combined with glycerol; and identification of polyphenols in 
glycerol extract by UPLC-Triple-TOF/MS (PDF)

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