Ultrasound-homogenization-assisted extraction of polyphenols from coconut mesocarp: Optimization study

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1. Introduction

Coconut (Cocos nucifera L.), an important monocotyledonous plant, belongs to the coconut genus in the Palmaceae, and widely grows in tropical and subtropical regions [1,2]. The structure of coconut fruit from the outside to the inside is the pericarp, testa, coconut kernel (solid endosperm), coconut water (liquid endosperm) and embryo. The mesocarp, called as coconut shell fiber, is composed of a large number of fibers inside the smooth exocarp. The endocarp, called as coconut shell, is the hard inner shell in the middle of coconut to protect the seed [3,4]. The coconut mesocarp (CM) and coconut endocarp (CE) are the main by-products of coconut production, accounting for 50% of the whole coconut. According to the latest statistics of FAO, global coconut production reaches 62.06 million tons in 2019 [5], about 30 million tons of CM and CE. These by-products degrade slowly and are often discarded as solid wastes on beaches and streets, causing serious environmental pollution [6,7]. At present, in addition to edible coconut water as a beverage and the kernel as the source food or oil, a small amount of CM and CE are used to produce activated carbon, carpets, ropes and agricultural fertilizers, etc. [8,9]. Recently, the biological activity and nutritional value of coconut pericarp have been studied. It is found that coconut pericarp is rich in phenolic compounds, such as catechin, epicatechin, tannin, coumaric acid and vanillic acid [2,10]. These phenolic compounds have been widely proved to have antibacterial, antiviral, anti-oxidant and anti-cancer activities [11–13].

High-efficiency extraction of natural active substances is the key process of Ultrasound-Assisted Extraction (UAE), Homogenization-Assisted Extraction (HAE), Homogenization-Ultrasound-Assisted Extraction (HUAE), and Ultrasound-Homogenization-Assisted Extraction (UHAE). The UAE and HAE are considered to be the main methods for efficient extraction of natural active ingredients. The former effectively destroys the cell wall structure and promotes the intermolecular diffusion based on the cavitation, thermal and mechanical effect of ultrasonic, while the latter breaks the material based on strong shear force between the rotor and stator. Their combinations (HUAE and UHAE) enhance the damage to the cell wall of raw materials and improve the extraction efficiency by the synergistic effect. The results showed that using 60% acetone (V : V) as extraction solvent, solid-liquid ratio of 1:5 g mL$^{-1}$, ultrasonic temperature of 80 °C, ultrasonic time of 80 min, ultrasonic power of 225 W, and then homogenizing at 10,000 rpm for 10 min, the total flavonoid content of CM reached the maximum value of 551.99 ± 12.69 mg Rutin g$^{-1}$ dry weight (dw), while the total phenolic content reached the maximum value of 289.48 ± 4.41 mg GAE g$^{-1}$ dw at 10,000 rpm for 5 min, which may be related to the oxidative degradation of polyphenols caused by the increase of polyphenol oxidase with the extension of homogenization time. This study provides a technical guarantee for the further utilization of phenolic substances in CM.
technology for intensive processing of raw materials, obtaining high value-added products, and realizing optimal utilization value. Compared with a single solvent (water, ethanol, methanol, acetone and ether), the simultaneous use of two or more solvents with different polarities can increase the extraction rate of phytochemicals [14,15]. For example, Chavan et al. [16] used different solvent systems to extract phenolics, tannins and sugars from beach pea (Lathyrus maritimus L.). As a result, the phenolics and condensed tannins extracted by the acetone-water system were more than those by ethanol–water or methanol–water system. Arivalagan et al. [2] found that adding water in the solvent system could improve the yield of phenolics in coconut tests. In addition, Ultrasound-Assisted (UAE) and Homogenization-Assisted Extraction (HAE) are considered to be the main methods for efficient extraction of natural active ingredients. The UAE is to use the cavitation, thermal and mechanical effect of ultrasonic to strengthen the treatment of materials [17–19], which effectively destroys the cell wall structure and promotes the intermolecular diffusion, resulting in fully release the active intracellular substances [20,21]. It has the advantages of short extraction time, less solvent consumption, and high extraction rate [22–23]. For instance, Rodiah et al. [3] found that compared with stirring extraction, UAE improved the yield of colorants in coconut mesocarp and exocarp, and the extraction time was short. However, to release the intracellular substances more fully, a stronger shear force is required. The HAE is to generate strong shear force, which makes the material molecules motion rate increases sharply, and reciprocate at high speed between the stator and rotor on the inner wall of the container, so that the material is split, broken and evenly dispersed in the medium [24,25]. Ke et al. [26] used a homogenate method to extract crude polysaccharides from Lentinus edodes with a yield of 13.2 ± 0.9%, which is 29.82% higher than the traditional heating extraction method, and has better DPPH scavenging activity. Regrettably, the long-term shearing action is easy to cause the oxidation, gelatinization, and the activity decreases of the substances. Among them, polyphenol oxidase (PPO) may increase with the increase of shear time, resulting in the oxidation of the extract substances, then decrease of their activity. PPO is a kind of highly efficient phenolic oxidase, which can catalyze the generation of catechol from monophenolic hydroxyl compounds under aerobic conditions, and also oxidize the dehydrogenation of catechol to quinone [27]. Finally, the quinone polymerizes and reacts with the amino acids in the cell, resulting in browning reaction, which affects the content of polyphenols and antioxidant activity of the product. Therefore, based on the advantages and disadvantages of the UAE and HAE can be combined to enhance the damage to the cell wall of raw materials by the synergistic effect, based on the less time and energy consumption. It has been reported that the anthocyanins and flavonols from bog bilberry (Vaccinium uliginosum L.) marc with carnosic acid as an antioxidant additive are extracted by Homogenization-ultrasound-assisted extraction (HUAEX), and the higher anthocyanins (13.95 ± 0.37 mg g⁻¹) and flavonols (3.51 ± 0.16 mg g⁻¹) were obtained [28]. In short, due to the lack of efficient extraction technology of CM and CE, the further utilization of their phenolic compounds is limited. Hence, optimizing the extraction process and improving the extraction rate are urgent problems. In this study, the effects of different solvents (water, methanol, 60% methanol, ethanol, 60% ethanol, acetone, 60% acetone) were evaluated and different extraction methods (UAE, HAE, Homogenization-Ultrasound-Assisted Extraction (HUAEX), and Ultrasound-Homogenization-Assisted Extraction (UHAE) on the extraction rate of phenolic compounds in CM and CE and were optimized. These results will provide a preliminary basis for the further application of CM and CE phenolic extracts.

2. Materials and methods

2.1. Materials and reagents

The CM and CE samples were provided by the Rubber Research Institute of the Chinese Academy of Tropical Agricultural Sciences. They were dried to constant weight in a drying oven (103 °C), then, the powder through a 50 mm sieves was collected and stored at – 4 °C for future analysis.

Catechol, citric acid, sodium hydroxide, methanol, acetone and all analytic-grade reagents were purchased from Macklin Biochemical Co. Ltd. (Shanghai, China).

### Table 1

| No. | Factor | Five level |
|-----|--------|------------|
| A   | B      | C          |
| 1   | 225    | 60         | 60         |
| 2   | 225    | 40         | 80         |
| 3   | 225    | 40         | 40         |
| 4   | 180    | 60         | 80         |
| 5   | 225    | 60         | 60         |
| 6   | 270    | 60         | 40         |
| 7   | 180    | 60         | 40         |
| 8   | 225    | 60         | 60         |
| 9   | 225    | 40         | 60         |
| 10  | 225    | 80         | 60         |
| 11  | 270    | 60         | 80         |
| 12  | 225    | 60         | 60         |
| 13  | 225    | 80         | 80         |
| 14  | 180    | 80         | 60         |
| 15  | 225    | 60         | 60         |
| 16  | 270    | 80         | 60         |
| 17  | 270    | 40         | 60         |

A: ultrasonic power (W), B: ultrasonic temperature (°C), C: ultrasonic time (min).

### Table 2

The experiment design and results of Response Surface Methodology.

| No. | Factor | TFC (mg Rutin g⁻¹ dw) |
|-----|--------|-----------------------|
| 1   | 225    | 382.86 ± 10.23        |
| 2   | 225    | 283.72 ± 11.51        |
| 3   | 225    | 301.21 ± 6.71         |
| 4   | 180    | 354.5 ± 18.65         |
| 5   | 225    | 398.43 ± 7.98         |
| 6   | 270    | 297.63 ± 6.16         |
| 7   | 180    | 352.25 ± 16.47        |
| 8   | 225    | 379.82 ± 8.35         |
| 9   | 225    | 351.17 ± 16.47        |
| 10  | 225    | 319.19 ± 18.82        |
| 11  | 270    | 302.71 ± 8.28         |
| 12  | 225    | 369.65 ± 10.21        |
| 13  | 225    | 408.56 ± 18.66        |
| 14  | 180    | 362.74 ± 8.40         |
| 15  | 225    | 365.46 ± 12.36        |
| 16  | 270    | 377.81 ± 13.66        |
| 17  | 270    | 278.89 ± 11.51        |

A: ultrasonic power (W), B: ultrasonic temperature (°C), C: ultrasonic time (min).

2.2. Determination of total phenolic content (TPC) and total flavonoid content (TFC)

According to Chen et al. [29], the TPC of the samples were determined by the Folin-Ciocalteu method, expressed as mg GAE g⁻¹ dry weight (dw) and the TFC of the samples were determined by the Aluminium salt colorimetry, expressed as mg Rutin g⁻¹ dw.

2.3. Solvent system

According to Li et al. [30] with minor modification, the basic extraction condition: The ultrasonic power of 360 W, the ultrasonic time of 30 min, the ultrasonic temperature of 60 °C and the solid–liquid ratio of 1:15 g mL⁻¹. Different solvents (water, methanol, methanol (60%, V_{methanol}V_{water}), ethanol, ethanol (60%, V_{ethanol}V_{water}), acetone, acetone (60%, V_{acetone}V_{water}) were used to extract phenolic compounds from the CM and CE.

2.4. Single factor experiment design of the UAE

Based on the optimum extraction solvent, the effects of ultrasonic temperature, ultrasonic time, solid–liquid ratio and ultrasonic power on TFC in CM and CE were investigated, respectively. The single factor experiment design was shown in Table 1.
3. Results and discussion

3.1. Solvent systems analysis

Solvents play a vital role in the extraction of phenolic compounds. Therefore, the effect of different solvents on the extraction rate of phenolic compounds in CM and CE were evaluated by measuring TFC and TPC in different solvent extracts. The results showed that the mixture of water and organic solvent had a higher extraction rate than single solvent. For example, the TFC of 60% acetone, 60% ethanol and 60% methanol were higher than water, anhydrous acetone, ethanol, and methanol (Fig. 1a). Especially, 60% acetone had the highest TFC extraction rate of CM (274.99 ± 7.92 mg Rutin g⁻¹ dw), while anhydrous acetone had the lowest extraction rate of CM (18.99 ± 1.00 mg Rutin g⁻¹ dw) (Fig. 1a). Meanwhile, the TFCs of CE showed a similar trend to those of CM, and were relatively lower than those of CM. The TFC of CE at 60% acetone was 21.65 ± 1.42 mg Rutin g⁻¹ dw, only accounting for 7.87% of CM. In addition, the change trend of TFC in CM and CE were like to that of TFC, and their highest TPCs were 196.98 ± 5.92 mg GAE g⁻¹ dw and 7.77 ± 0.85 mg GAE g⁻¹ dw at 60% acetone (Fig. 1b). In previous studies, Zhao et al. [32] reported that the TPCs of three Chinese barley varieties (Ken-3, K4B, and Gan-3) were extracted using four solvent (80% acetone, 80% ethanol, 80% methanol, water). Their TPC values varied from 1.03 (water extract from Ken-3 barley) to 1.87 mg GAE g⁻¹ dw (80% acetone extract from Gan-3 barley). Saifullah et al. [33] also reported the effect of five solvents on the extraction yields of phenolic compounds and antioxidant properties of lemon scented tea tree leaves. The results showed that 50% acetone (60.24 ± 2.84 mg GAE g⁻¹ dw) > 50% ethanol (35.11 ± 2.84 mg GAE g⁻¹ dw) > water (22.46 ± 3.68 mg GAE g⁻¹ dw) > acetone (7.99 ± 0.85 mg GAE g⁻¹ dw) > ethanol (7.35 ± 1.03 mg GAE g⁻¹ dw). On the whole, the extraction solvent had a significant effect on the extraction rate of phenolics, and acetone–water system had obvious advantages.

3.2. Single factor experiment analysis of the UAE

Using 60% acetone as extraction solvent, the effects of ultrasonic temperature, ultrasonic time, solid-liquid ratio and ultrasonic power on the extraction rate of phenolic compounds in CM and CE were studied.
Under the same basic conditions, single factor experiments were carried out at five levels. As shown in Fig. 2a, b, d, with the increase of ultrasonic temperature, ultrasonic time and ultrasonic power, the TFC of CM and CE initially increased and then decreased. The increase of TFC may be the destruction of the surface of the solid matrix, which makes it easier for the solvent to penetrate into the solid matrix and increases the solubility of the solute. When the ultrasonic power, temperature and time reach a certain threshold, the cell wall rupture caused by the ultrasonic effect tends saturated. However, the extensive ultrasound degraded and destroyed phenolic compounds extracted from CM and CE, resulting in the thermal degradation of phenolic compounds [19]. Previous reports on the yield of phenolic compounds extracted from olive fruits using the UAE also demonstrated increase of yield with increasing time from 4 to 20 min, whereas prolonged treatment of ultrasound after 20 min declined the yield [34]. Therefore, after reaching a certain threshold, the TFC does not increase but decrease. Additionally, with the increase of the solid–liquid ratio, the TFC showed a downward trend in CM and an unchanged trend in CE (Fig. 2c). The high ratio reduces the recovery rate and increases the energy and reagents consumption. Thus, the ultrasonic power, time and temperature will be further optimized by response

![Fig. 2. Effects of ultrasonic temperature (a), ultrasonic time (b), solid–liquid ratio (c) and ultrasonic power (d) on TFC CM: coconut mesocarp, CE: coconut endocarp.](image)

| Source          | Sum of Squares | df | Mean Square | F-value | P-value | Significant |
|-----------------|----------------|----|-------------|---------|---------|-------------|
| Model           | 25235.55       | 9  | 2803.95     | 12.13   | 0.0017  | **          |
| A- ultrasonic power | 3346.44       | 1  | 3346.44     | 14.48   | 0.0067  | **          |
| B- ultrasonic temperature | 8020.74      | 1  | 8020.74     | 34.71   | 0.0006  | **          |
| C- ultrasonic time | 784.28        | 1  | 784.28      | 3.39    | 0.1080  |             |
| AB              | 1907.51        | 1  | 1907.51     | 8.25    | 0.0239  |             |
| AC              | 2.00           | 1  | 2.00        | 0.0087  | 0.9284  |             |
| BC              | 2854.76        | 1  | 2854.76     | 12.35   | 0.0098  | **          |
| A^2             | 1519.12        | 1  | 1519.12     | 6.57    | 0.0373  |             |
| B^2             | 1303.81        | 1  | 1303.81     | 5.64    | 0.0492  |             |
| C^2             | 4718.78        | 1  | 4718.78     | 20.42   | 0.0027  | **          |
| Residual        | 1617.62        | 7  | 231.09      |         |         |             |
| Lack of Fit     | 954.07         | 3  | 318.02      | 1.92    | 0.2683  |             |
| Pure Error      | 663.55         | 4  | 165.89      |         |         |             |
| Cor Total       | 26853.18       | 16 |             |         |         |             |

*a* Indicates significant difference $P < 0.05$, ** indicates significant difference $P < 0.01$.
surface methodology (RSM), except that the material-liquid ratio is 1 : 5 g mL⁻¹ to minimize energy consumption.

3.3. Response surface experiment analysis of the UAE

Considering the high TPC and TFC in CM, the optimization experiment took CM as an example. According to the results of single factor experiment, the three factors and three levels was carried out by RSM. A total of 17 experiments were designed, including 5 central point experiments shown in Table 2. The data were fitted by multiple regression with Design-Expert software, and the quadratic multiple regression equation was obtained. The ultrasonic power (A), ultrasonic temperature (B) and ultrasonic time (C) were taken as independent variables, and the TFC was taken as the response value. The regression equation (Eq. (2)) was as follows: TFC (mg Rutin g⁻¹ dw) = 379.24−20.45 × A + 31.66 × B + 9.90 × C + 21.84 × AB + 0.7075 × AC + 26.72 × BC − 18.99 × A² − 17.60 × B² − 33.48 × C². (2)

From the results of variance analysis in Table 4, the regression equation of the model had significant difference (P < 0.01), and significantly affected of ultrasonic power (A) and temperature (B). The reciprocal action of AB and BC displayed a stronger influence on TFC values, but the reciprocal action of AC was shown to be insignificant. Furthermore, the second-order reciprocal action of A², B² and C² had significant effects on TFC values, and compared with A² and B², C² had more significant effects. The reliable predictive value of the model (R² = 0.9398) supported its good predictability for the response variables. The adjusted coefficient of determination was Rₐ² = 0.8623, suggesting that the model reflected 86% of the data and had a good fitting degree. The “Lack of Fit F-value” of 1.92 implied that the model equation was sufficient to predict the extraction rate of TFC in CM within the range of experimental variables.

According to the regression equation, the shape of the fitted response surface was investigated, and the interaction of various factors on the TFC in CM was analyzed. As shown in Fig. 3, The influence of each variable on TFC showed a trend of initially increased and then decreased, and the steeper the slope of the response surface, the more significant the interaction [35]. With the change of ultrasonic power (A) and temperature (B), the response surface tended to be parabola, and the slope of the surface was steep, indicating that there was an interaction between A and B in Fig. 3a, which was consistent with those of ANOVA (Pₐₙ < 0.05). Similarly, there was an interaction between B and C (Pₐₙ < 0.01) (Fig. 3c). However, the curved surface was flat (Fig. 3b), indicating that the interaction between ultrasonic power (A) and ultrasonic time (C) was not significant (Pₐₙ > 0.05).

Based on the Design-Expert software prediction, the optimum extraction process of TFC in CM was as follows: ultrasonic power of 225.11 W, ultrasonic temperature of 80 °C, ultrasonic time of 70.94 min, and the maximum TFC was 416.636 mg Rutin g⁻¹ dw. Considering the convenience and feasibility of practical operation, the process was adjusted as follows: ultrasonic power of 225 W, ultrasonic temperature of 80 °C, ultrasonic time of 80 min and the practical value was 408.56 mg ± 18.66 Rutin g⁻¹ dw. These differences indicated that the optimum extraction process was related to the properties of plant materials.

3.4. HAE experiment analysis

To evaluating the effect of the combination of UAE and HAE on the extraction rate of phenolic compounds from CM, the optimal UAE (ultrasonic power of 225 W, ultrasonic temperature of 80 °C, ultrasonic time of 80 min) and the worst UAEx (ultrasonic power of 270 W, ultrasonic temperature of 60 °C, ultrasonic time of 40 min) and HAE (0, 2.5, 5.0, 7.5, and 10 min) at 10,000 rpm were selected. The results showed that compared with the TFC of 60% acetone (266.99 ± 4.45 mg
Rutin g\(^{-1}\) dw), the TFCs of UAEo, UAEw and HAE were 408.56 ± 18.66, 297.63 ± 4.04 and 309.48 ± 7.85 mg Rutin g\(^{-1}\) dw, respectively. The TFC was enhanced to 514.59 ± 21.02 mg Rutin g\(^{-1}\) dw by HUAEo and 551.99 ± 12.69 mg Rutin g\(^{-1}\) dw by UHAEo (Fig. 4a). Under the same homogenization time in all combinations, the TFCs of UHAEo and UHAEw were higher than those of HUAEo and HUAEw (Fig. 4), respectively. Moreover, with the increase of homogenization time, TFC showed an upward trend from 408.56 ± 18.66 to 551.99 ± 12.69 mg Rutin g\(^{-1}\) dw under UHAEo and 297.63 ± 4.04 to 385.81 ± 10.69 mg Rutin g\(^{-1}\) dw under UHAEw. These results indicated that ultrasound before homogenization is more conducive to the release of phenolics, and they increases with the prolongation of homogenization time.

However, the change trend of TFC was slightly different from that of TPC. By contrast, TPC initially increased (0 to 5 min) and then decreased (5 to 10 min) (Fig. 5). This initial increase can damage cell wall or cell structure by homogenization, the TPCs of UHAEo and UHAEw were higher than those of HUAEo and HUAEw (Fig. 4), respectively. Moreover, with the increase of homogenization time, TFC showed an upward trend from 408.56 ± 18.66 to 551.99 ± 12.69 mg Rutin g\(^{-1}\) dw under UHAEo and 297.63 ± 4.04 to 385.81 ± 10.69 mg Rutin g\(^{-1}\) dw under UHAEw. These results indicated that ultrasound before homogenization is more conducive to the release of phenolics, and they increases with the prolongation of homogenization time.

In this study, the extraction process of phenolic compounds from CM and CE was optimized. Based on 60% acetone as the optimum solvent, the UAE of TFC was further optimized by single factor experiment and three-factor and three-level response surface design. With solid–liquid ratio of 1:5 g mL\(^{-1}\), ultrasonic temperature of 80 °C, ultrasonic time of 80 min and ultrasonic power of 225 W, the TFC reached the maximum of 408.56 ± 18.66 mg Rutin g\(^{-1}\) dw. Then compared with the four process systems of UAE, HAE, UHAE and HUAE, the TPC with UHAE (10 min, high-pressure homogenization. To track whether PPO increases with homogenization time, the contents of PPO in the extract at 0, 2.5, 5, 7.5 and 10 min were investigated (Fig. 6). The results showed that the PPO in CM gradually increased with increasing homogenization time. Therefore, long-term homogenization can promote the release of PPO in cell tissues, resulting in the decrease of TPC in the system. Orak also found that there was a negative correlation between PPO and TPC (R = −0.390) [38]. Furthermore, there was no significant difference between HUAE and UHAE on the extraction rate of TPC. Therefore, exploring the specific extraction process of CM can maximize the extraction efficiency and lay a good foundation for the further study of effective components.

**4. Conclusion**

The extraction of phenolic compounds from CM and CE was optimized. Based on 60% acetone as the optimum solvent, the UAE of TFC was further optimized by single factor experiment and three-factor and three-level response surface design. With solid–liquid ratio of 1:5 g mL\(^{-1}\), ultrasonic temperature of 80 °C, ultrasonic time of 80 min and ultrasonic power of 225 W, the TFC reached the maximum of 408.56 ± 18.66 mg Rutin g\(^{-1}\) dw. Then compared with the four process systems of UAE, HAE, UHAE and HUAE, the TPC with UHAE (10 min, high-pressure homogenization. To track whether PPO increases with homogenization time, the contents of PPO in the extract at 0, 2.5, 5, 7.5 and 10 min were investigated (Fig. 6). The results showed that the PPO in CM gradually increased with increasing homogenization time. Therefore, long-term homogenization can promote the release of PPO in cell tissues, resulting in the decrease of TPC in the system. Orak also found that there was a negative correlation between PPO and TPC (R = −0.390) [38]. Furthermore, there was no significant difference between HUAE and UHAE on the extraction rate of TPC. Therefore, exploring the specific extraction process of CM can maximize the extraction efficiency and lay a good foundation for the further study of effective components.
10,000 rpm) reached the highest of 551.99 in PPO among HAE, HUAE and UHAE (CM (e)) indicate significant differences in PPO among different extraction methods of substances in CM.

Long time homogenization would promote the release of phenolic compounds with antioxidant potential from coconut (Cocos nucifera L). (2020-101).

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