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Various Environmentally Friendly Co-Extraction Methods of Polysaccharides and Polyphenols from Mangosteen Peels (*Garcinia Mangostana* L.)

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In this study, various environmentally friendly approaches such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and enzyme-ultrasound assisted extraction (EUAE) were investigated to simultaneously separate polysaccharides and polyphenols from mangosteen fruit peels (MPs). The yield of polysaccharides (PSY) and total phenolic content (TPC) were computed to compare the efficiency among methods. The chemical structure through Fourier-transform infrared (FTIR) spectrophotometry of the extracted polysaccharides as well as the antioxidant properties of the phenolic extracts were also determined. The result reveals that EUAE at the optimal condition (enzyme content of 6.6 U/g, enzymatic incubation time of 60 min, pH of 5.0 and ultrasonic duration of 30 min) provided much higher PSY (32.65%) and TPC of (107.8 mg gallic acid/ g of dry weight of sample) than MAE and UAE. The FTIR spectrum of the EUAE polysaccharides were remarkably distinct with those of MAE and UAE ones, indicating the differences in chemistry of the polysaccharides to be extracted. The findings of the study indicate that the polysaccharides and polyphenols from MPs by EUAE treatment can be used as natural ingredients in pharmaceutical and functional food formulations.

1. Introduction

Mangosteen (*Garcinia mangostana L.*) is a tropical fruit that has gained significant recognition for its traditional medicine use in Southeast Asia. It contains a significant amount of waste which can be utilized as pharmaceutical raw materials. The main waste of mangosteen fruits is the rind, which contains various bioactive compounds such as phenolic acids, anthocyanins, xanthones, providing therapeutic potentials in traditional medicine such as fever treatment and inflammation. These biological compounds have also been investigated for several pharmacological properties, like anti-inflammatory and anti-tumor activities. In addition, mangosteen peels also contain a remarkable amount of polysaccharides (Rohman et al., 2019; Wathoni et al., 2019) In recent years, there has been a growing focus on polysaccharides from fruits due to their biocompatible, biodegradable, and non-toxic characteristics. Consequently, they are gaining popularity in the development of edible food packaging materials, offering desirable transparency, mechanical strength as well as lipid and gas barrier properties (Cruz-Monterrosa et al., 2023).Conventionally, the extraction of polysaccharides and phenolics from mangosteen peels has relied on the use of various solvents, sometimes in combination with heat, as the conventional approach. However, this method presents limitations including prolonged extraction duration, specific solvent prerequisites, limited solvent recovery efficiency and the thermal breakdown of desired compounds (Wen et al., 2020) In order to address these obstacles, it is imperative to seek innovative environmentally friendly techniques that can reduce the extraction time, minimize the solvent usage and enhance the yield and quality of the extracts. Recently, enzyme assisted extraction (EAE) and ultrasound-assisted extraction (UAE) are recognized as “green technologies” since they meet the requirements set by the Environment Protection Agency in the USA. The EAE employs water as solvent instead of organic chemicals

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for extraction of bioactive compounds from pomelo peels (Nguyen et al., 2021) and polysaccharides from pomegranate seeds and peels (Zhai et al., 2018). Similarly, the UAE has been utilized as an environmentally friendly and cost-effective method, serving as an alternative to traditional procedures, for extracting several bioactive components and polysaccharides from fruits and vegetables. Meanwhile, microwave-assisted extraction (MAE) is a method that utilizes microwave energy to separate target compounds from the matrices. This technique offers numerous benefits, including high efficiency, low energy consumption, quick processing time, cost-effectiveness, and minimal solvent usage (Tran et al., 2023). Several studies have focused on the polysaccharides and phenolics of mangosteen fruits, with each component extracted individually (Rohman et al., 2019; Wathoni et al., 2019). As agricultural residue production has expanded globally and there has been a lot of interest in the concurrent production of several products from fruits wastes. However, to the best of our knowledge, there is currently no information available on the co-extraction of polysaccharides and phenolics from mangosteen peels using various methods. Consequently, this study aims to fill this gap by investigating different environmentally friendly co-extraction processes on these two components.

2. Materials and methods

2.1 Materials

The dried mangosteen peels (MPs) were collected from an organic farm at Ben Tre province, Vietnam. The moisture content of MPs was approximately 10%, the dried MPs were powered and sieved at 250 µm. The fine powder was stored at room temperature. Chemicals used in the study included sodium acetate, acetic acid, citric acid, sodium carbonate, ethanol (from Xilong), DPPH and gallic acid (from Acros), Folin-Ciocalteu reagent, quercetin and cellulase Onozuka R-10 from *Trichoderma viride* (from Sigma – Aldrich).

2.2 Extraction methods

2.2.1 Microwave-assisted extraction (MAE)

Polysaccharides and phenolics were extracted from dried MPs using acidified water as solvent (Tran et al., 2023). In detail, 1 g of MPs was mixed with 30 mL citric acid solution (pH 2.0) and then microwaved (R-21A1(S)VN) at different power levels of 80, 240, 400, 560 and 800 W when the duration was kept at 20 min. The extraction time was then experimented in the range of 5 to 30 min at the optimal microwave power. Afterward, the obtained solution was centrifuged at 4500 rpm for 20 min (Mikro 220R, Hettich, Germany). The supernatant was added with absolute ethanol at a ratio of 1:10 (v/v) to precipitate polysaccharides. The resultant mixture was then kept at a 4^{o}C for 24 h. After that, the precipitates were filtered and collected to determine the yield of polysaccharides while the remaining ethanolic solution was utilized to determine total phenolic content (TPC)

2.2.2 Ultrasound-assisted extraction (UAE)

Similar to MAE, the mixtures of MPs and acidified water were prepared. However, they were then treated inside an ultrasonic bath (WUC-A10H, Daihan, Korea) at 40 kHz with the duration of sonication from 30 to 70 min at the fixed temperature of 60^{o}C. The bath temperature was then experimented at 30 to 80^{o}C at the found optimal ultrasonic time. After treatment, there were separated from the solutions as detailed in section 2.2.1

2.2.3 Enzyme -ultrasound assisted extraction (EUAE)

Briefly, 1 g of MPs was mixed with 30 mL sodium acetate buffers of pH 4.5 containing different enzyme contents (2.2, 4.4, 6.6, 8.7, 10.9, 13.1, 15.3 and 17.5 U/ g MPs). The enzymatic treatment was carried out at 50^{o}C for 30 min in a shaking incubator at 115 rpm. The next factor to be investigated was the pH value in the range of 3 to 7. Subsequently, the incubation duration was studied at 30 to 150 min. After that, the mixture was heated to 90^{o}C in a water bath for 5 min to inactive enzyme. The resultant mixtures were then ultrasonicated at 50^{o}C from 20 to 60 min. Upon completing this procedure, polysaccharides and phenolics were recovered as section 2.2.1

2.3 The yield of polysaccharides and total phenolic content

The obtained wet polysaccharides were dried at 50^{o}C until reaching a constant weight. The PSY was calculated based on the dried polysaccharides and mangosteen peel powder (Tien et al., 2022). In addition, the total phenolic content (TPC) was determined by the method (Tien et al., 2022). TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram sample.

2.4 Total flavonoid content (TFC) and antioxidant activity of the phenolic extract

The total flavonoid content (TFC) expressed as mg quercetin equivalent (QE) per mL extract and the antioxidant activity was evaluated through DPPH free radical scavenging capacity according to (Tien et al., 2022)

2.5 Chemical structure of the obtained polysaccharides

The chemical structure of obtained polysaccharides was determined by using an FT/IR – 4700 spectrophotometers (Jasco, Japan). The wavenumber of 4000 – 400 cm^{-1} was scanned with the resolution of 1 cm^{-1}.

2.6 Statistical analysis

Triplicates of each experiment were carried out. The experimental results were presented as mean ± SD (standard deviation). Analysis of variance was performed using the Minitab software ver. 19 at the level of confidence of 95%.

3. Results and discussion

3.1 The impact of MAE on the PSY and TPC

Figures 1A and 1B illustrate the effects of different microwave powers and treatment durations on PSY and TPC during MAE process, respectively. Figure 1A indicates that lower power levels (80 and 240 W) may not generate sufficient heat to fully disintegrate the cell wall to extract the substances. Hence, the extraction capacity for polysaccharides and phenolics from MPs exhibited a progressive increase when the microwave power was raised up to 400 W. The highest values of PSY (19.57%) and TPC (83.04 mg GAE/ g DW) were both achieved at the power level of 400 W. However, thereafter, higher microwave powers showed negative impacts, especially at 800 W. However, excess power levels could lead to overheating and subsequently reducing the efficiency of the extraction process. Therefore, it was determined that the power level of 400 W was appropriate for the extraction process in this study. The duration of the extraction process is another critical parameter in the extraction of polysaccharides and phenolics from plant materials using MAE. To examine its effect on PSY and TPC, experiments were conducted in various extraction durations (5 to 30 min), and the results are shown in Figure 1B. From the observations, the PSY (19.98%) and TPC (82.79 mg GAE/ g DW) reached their maximum values when the irradiation time was 20 min, after this point, they reduced significantly.

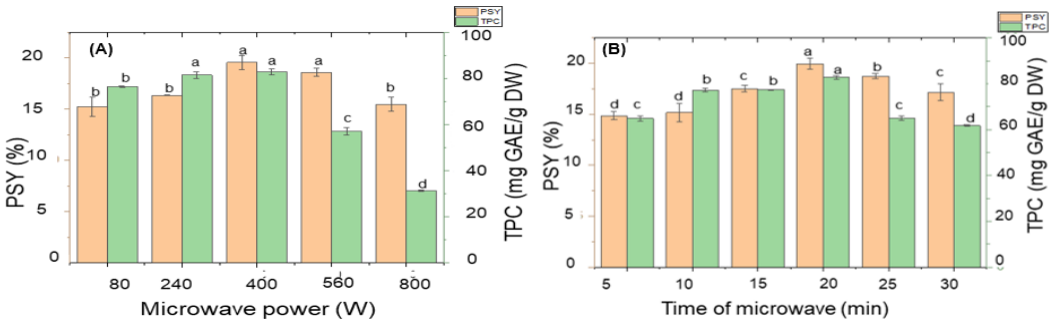

Figure 1. Effects of MAE on PSY and TPC. Different letters indicate significant differences among same color sample ($p < 0.05$).

3.2 The impact of UAE on PSY and TPC

Figures 2A and 2B display the effects of extraction time and temperature of UAE on the PSY and TPC, respectively. Figure 2A indicates that the highest PSY (13.90 to 14.33%) and TPC (89.62 to 92.26 mg GAE/ g DW) were obtained at 30 and 40 minutes of ultrasonication, after that both decreased over time. The increases in the extraction yields during the initial period were due to the cavitation effect of ultrasound, which in turn increased the swelling, hydration, fragmentation, and pore formation within the plant tissue matrix (Le & Le., 2021) These changes contributed to the increased exposure of the target solutes with the extraction medium, hence enhancing their subsequent release into the solvent. However, after long exposure over time, degradation of the extracted compounds may happen, leading to their yield reductions at the later period. Therefore, the suitable sonication time of 40 min was used for the next experiment. In the experiment on the effect of sonication temperature, Figure 2B reveals that the PSY and TPC increased with the rising ultrasonic temperature and reached their peak values at 50°C, which were 16.30 % and 87.50 mg GAE/g DW, respectively for PSY and TPC. This was due to the fact that high temperatures could decrease the viscosity of the extraction solvent, and hence increasing its diffusivity within the tissue matrix, facilitating the contact of polysaccharides and phenolics with the solvent and their consequent release into the solvent (Zhang et al., 2017). However, extremely high ultrasonic temperatures in the range of 60 – 80°C may lead to the degradation of polysaccharides and phenolics

which were not advantageous to their extraction yields. In this study, the ultrasonic temperature of 50°C was therefore suitable for UAE process.

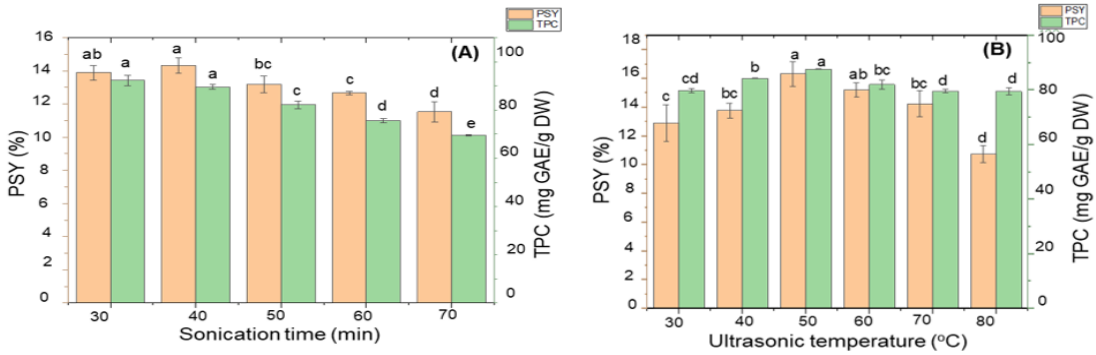

Figure 2. Effects of UAE on PSY and TPC. Different letters indicate statistically significant differences among samples with the same color ($p < 0.05$)

3.3 The impact of EUAE on PSY and TPC

Figures 3A to 3D present the effects of enzyme content, pH, enzymatic incubation time and ultrasonic time on the PSY and TPC from EUAE, respectively. Figure 3A indicates that the highest PSY was obtained in the enzyme content range of 6.6 to 13.1 U/g where their values were not significantly different among samples ($p > 0.05$). Meanwhile, the TPC reached its peak at the enzyme content of 6.6 U/g. The plant cell wall is a firm framework of cellulose, which is embedded inside a gelatinous matrix consisting of pectic substances, hemicellulose, and glycoprotein (Al-Dhabi et al., 2017). Therefore, the utilization of specific enzymes (i.e. cellulase in this study) could facilitate the cell wall degradation and the hydrolysis of structural matrices to release the target compounds. With a small amount, enzyme molecules may not have enough interaction with their substrates to provide adequate efficiency. On the other hand, an excess amount of enzyme could lead to the degradation of extracted products or a higher processing cost. This finding indicated that the cellulase dose of 6.6 U/g was sufficient to obtain the high values of PSY and TPC from MPs. Moreover, the efficiency of enzymatic treatment could be influenced by pH due to the existence of optimal pH values for different enzymes. As observed from Figure 3B, the PSY and TPC values enhanced with increasing pH values from 3 to 5

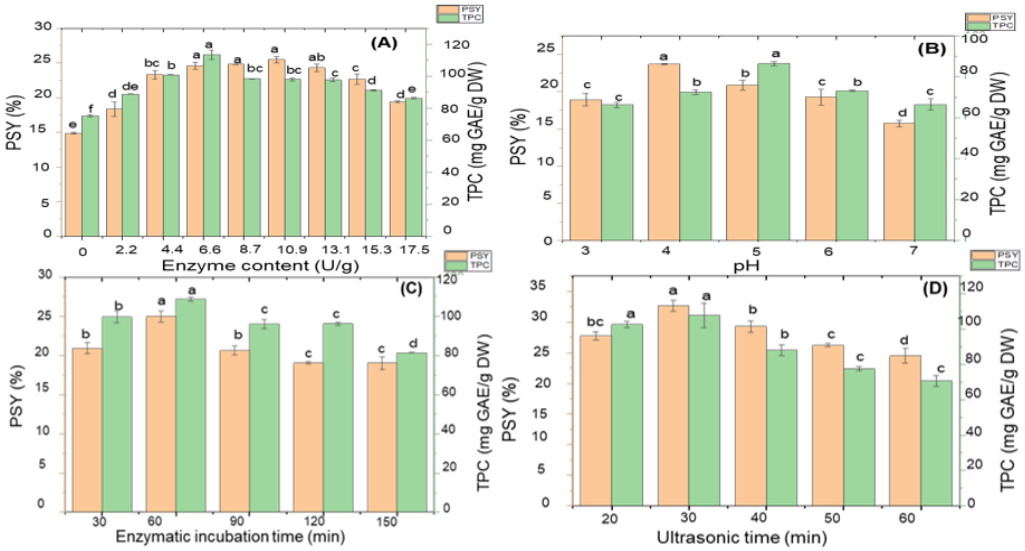

Figure 3. Effects of EUAE on PSY and TPC. Different letters indicated statistically significant differences among samples with the same color ($p < 0.05$)

However, the higher pH levels (> 5) could have a detrimental effect on the extraction of polysaccharides and phenolics. This phenomenon could be attributed to the potential impact of pH on the active sites or functional groups within the enzyme's conformation, which can be modified or regulated by modifying pH value (Al-Dhabi et al., 2017). Based on the obtained results, the pH value of 5 was considered suitable and used for further

experiments. In addition, duration was found to be a significant factor in an enzymatic treatment that affects the yields of polysaccharides and phenolics. Figure 3C indicates that the PSY and TPC values reached their peaks of 25% and 108.93 mg GAE/g DW at 60 min, respectively. After this period, their values tended to decrease gradually. This could be since the longer time could lead to the hydrolysis or modification of the extracted polysaccharides and phenolics by cellulase. Therefore, the suitable enzymolysis time for the extraction was 60 min. To further increase the extraction yields, the ultrasonic treatment was subsequently applied at 50°C (the suitable temperature found in Section 3.2) at different durations from 20 to 60 min. Figure 3D reveals that the successive ultrasonic treatment further increased the PSY and TPC to 32.65% and 110.86 mg GAE/ g DW, respectively with the suitable treatment time of 30 min. This implies that the employment of ultrasound in the later stage after enzymatic treatment could further break down certain interactions and bonds in the cell matrix that had not been hydrolyzed by enzyme to release the target substances. Furthermore, ultrasound could also increase the swelling and softness of cell walls (Abou-Elseoud et al., 2021) that could facilitate the solubilization of released polysaccharides and phenolics into the solvent.

3.4 Chemical structure of the extracted polysaccharides

Figure 4 reveals that MAE and UAE produced polysaccharides with a similar structure and hence a parallel FTIR pattern, where EUAE created a significant difference in the spectrum, especially in the wavelength range of 1200 to 1800 cm^{-1}. However, three of the polysaccharides had a similar broad peak at 3000-3600 cm^{-1}, which was assigned for the bending vibration of hydroxyl groups, typically abundant in the polysaccharide structure. In addition, the peak at around 2915 cm^{-1} was attributed to the absorption related to CH, CH_2 and CH_3 groups or methyl ester in the structure of galacturonic acid (Tien et al., 2022). The absorbance region of several peaks in the wavelength range of 900-1200 cm^{-1} was associated with the fingerprint structure of most polysaccharides, commonly related to C-O and C-C stretching (Liu et al., 2021) with little obvious variation during various extraction procedures. On the other hand, the distinct peaks at 1714 and 1613 cm^{-1} in the polysaccharides extracted by MAE and UAE but absent in the one extract by EUAE were the typical absorbance of C=O stretching and COO- stretching in the structure of pectin, which were commonly used to determine its degree of esterification. In addition, their peaks at 1211 cm^{-1} may be related to the structure of galacturonic acid with the absence of glycosidic bonds. Meanwhile, the EUAE polysaccharide had unidentical peaks in this region, where the peak assignments may include 1630 and 1536 cm^{-1} for carbonyl groups, 1605 cm^{-1} for COO^- asymmetric stretching and 1403 cm^{-1} for COO^- symmetric stretching in the complex network structure of rhamnogalacturonan, homogalacturonan and/or polygalacturonic acid (Liu et al., 2021)

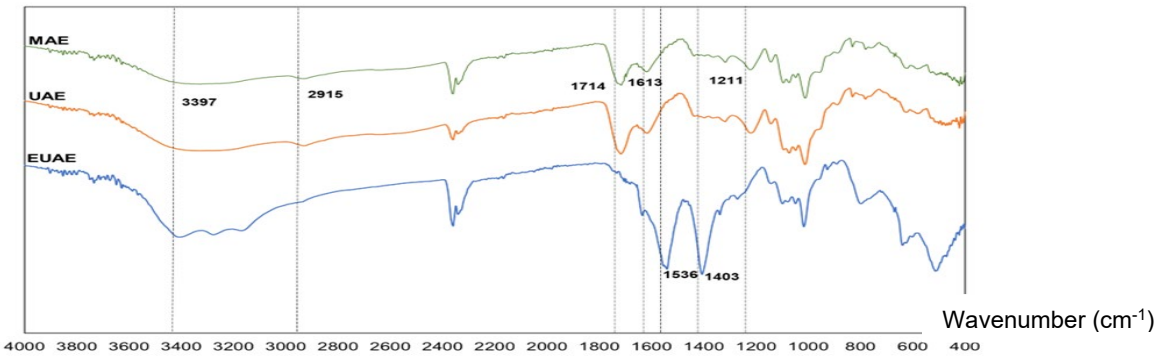

Figure 4. FTIR spectra of the polysaccharides obtained from different extraction methods

3.5 Antioxidant properties of the phenolic extracts

The phenolic solutions after extraction were concentrated into thick extracts to evaluate their potential antioxidant properties through the total flavonoid content (TFC) and DPPH free radical scavenging activity. Table 1 illustrates that EUAE produced the phenolic extract with much higher value of TFC (135.6 mg QE/ mL extract), 3.7-fold and 5.2-fold higher than those of the extracts from MAE and UAE. This value was considered equivalent or higher than those reported for other plant materials such as the extracts from *Grewia carpinifolia* (13.2 mg QE/g extract) (Adebiyi et al., 2017) and *Algerian Ficus carica* (14.1 mg QE/ g extract) (Mahmoudi et al., 2016) which implying the potential of MPs extract in practical applications. Aligning with the results of TFC, Table 1 also reveals that the EUAE phenolic extract exhibited much stronger antioxidant capacity, 94.4% in scavenging DPPH free radicals, which was approximate 3-fold higher than those of MAE and UAE extracts. This suggests that flavonoid compounds may have a major contribution to the antioxidant properties of MPs.

Table 1: Effects of different extraction methods on PSY, TPC and antioxidant properties of MPs

Method	PSY (%)	TPC (mg GAE/g DW)	TFC (mg QE/mL extract)	DPPH (%)
MAE	19.98 ± 0.55^{b}	82.8 ± 0.7^{b}	36.3 ± 0.4^{b}	32.4 ± 1.9^{b}
UAE	16.30 ± 0.85^{c}	87.5 ± 0.2^{b}	26.5 ± 0.5^{c}	29.7 ± 1.5^{b}
EUAE	32.65 ± 0.92^{a}	107.8 ± 7.0^{a}	135.6 ± 2.6^{a}	94.4 ± 0.2^{a}

Different superscripts indicate significant differences in the same column ($p < 0.05$)

4. Conclusion

In this study, the effects of MAE, UAE and EUAE techniques on PSY and TPC were investigated in-depth. The results showed that the EUAE resulted in the highest values of PSY and TPC among three methods. FTIR curves confirmed the chemistry of polysaccharides in three samples, but the chemical structure of EUAE polysaccharides were distinguished from two others. The phenolics extract of EUAE also displayed high antioxidant activities, implying its potential applications in food and pharmaceuticals.

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