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Ultrasonic-assisted Processes to Recover Phenolics from Watermelon Rinds

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A combination of Ultrasonic-Assisted Extraction (UAE) and Natural Deep Eutectic Solvent (NADES) was employed to recover phenolic compounds from watermelon rind powder. Among the various NADES formulations tested, the most efficient formulation in terms of extraction effectiveness, as determined by Total Phenolic Contents (TPC), was the one derived from lactic acid and 1,2–propanediol. Single-factor experiments were systematically conducted to evaluate the impact of different UAE parameters, including the liquid-to-solid ratio, ultrasonic power, water content in NADES, and extraction duration, on TPC. The favourable conditions for the UAE process using the lactic acid and 1,2–propanediol-based NADES were found to be as follows: a liquid-to-solid ratio of 60 ml/g, 20% water content in NADES, a temperature of 30 °C, and an extraction duration of 15 minutes. This research presents an efficient, environmentally friendly, and practical method for the retrieval of phenolic compounds from watermelon rind powder.

1. Introduction

Watermelon (Citrullus lanatus), which accounted for approximately 109 million tonnes of global production in 2013 (Faostat, 2016), holds the distinction of being the largest cultivated fruit crop worldwide. It is acknowledged for its contribution as a reservoir of vitamins (A, B, C, and E), essential mineral salts (K, Mg, Ca, and Fe), unbound amino acids (citrulline and arginine), carotenoid pigments (primarily lycopene), and phenolic components (Tlili et al., 2011). Notably, the watermelon rind constitutes roughly one-thirdof the complete fruit mass (Kumar, 1985), a segment typically discarded despite its suitability for consumption (Al-Sayed and Ahmed, 2013). Improper disposal of these peels leads to environmental pollution and the need for waste management. Nonetheless, these rinds hold significant promise as a reservoir of advantageous bioactive constituents like amino acids, alkaloids, carotenoids, phenolics, and flavonoids, renowned for their capacity to counter the effects of free radicals (Petkowicz et al., 2017, Wehner et al., 2001). Free radicals exert deleterious impacts on vital macromolecules such as proteins, lipids, and DNA, contributing to the onset of chronic disorders such as cardiovascular ailments, respiratory issues, cancer, neurodegenerative diseases, and gastrointestinal disorders (Corrêa et al., 2016). The inherent natural compounds within passion fruit peels function as scavengers, nullifying free radicals and interrupting the oxidative cascades that inflict harm upon the body's organs. Consequently, it becomes imperative to establish a suitable extraction technique to effectively retrieve these invaluable bioactive components from watermelon rinds.

Extraction is a critical step in the recovery of bioactive compounds. The traditional extraction techniques, including solvent extraction, and mechanical expelling are commonly utilized to recover plant component. The limitation of solvent extraction is the enormous usage of solvents, while that of mechanical expelling is low recovery yield. (Kumar et al., 2021). The disadvantages of other modern extraction techniques, such as supercritical fluid extraction and microwave-assisted extraction demands substantial capital investment and the presence of an aqueous phase, respectively Ultrasonic-assisted extraction (UAE) operates by utilizing ultrasonic waves to induce cavitation in the extraction medium (Le et al., 2022, Mathialagan et al., 2017). The cavitation phenomenon occurs when intense rarefaction cycles cause molecules within the medium to be pushed apart, leading to the formation of cavitation bubbles. These bubbles subsequently implode, disrupting cell walls. This

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disruption of cell walls promotes the penetration of solvents into the material resulting in an improved extraction yield of bioactive compounds (Kumar et al., 2021). Ultrasonic-assisted extraction (UAE) is considered an environmentally friendly technology due to its capacity to achieve high extraction yields while minimizing energy, time, and solvent consumption (Kumar et al., 2021). UAE has proven effective in boosting the recovery of bioactive compounds from plants, as demonstrated in previous studies (Amiri-Rigi et al., 2016, Moro et al., 2018). There were no studies extracting phenolics from watermelon rinds using the combination of UAE and NADES. Furthermore, previous research has employed an organic solvent for extracting these compounds, whereas our study utilized a bio-based solvent to enhance extraction efficiency.

The objective of this research was to employ ultrasonic waves for the extraction of phenolic compounds from watermelon rinds. Eight different solvent formulations were prepared to assess their influence on the efficiency of phenolic compound recovery. The study also explored the impact of various UAE parameters on the extraction yield of phenolic compounds. Response surface methodology (RSM) is used to optimize the UAE combined with the NADES.

2. Material and Methods

2.1 Materials

Watermelon rinds were sourced from Nam Viet Company, situated in Di An, Binh Duong, Vietnam. Subsequently, these rinds were dehydrated at a temperature of 45°C until their moisture content reached 5%. The desiccated rinds were then subjected to milling to produce watermelon rind powder (WP). The following chemicals and reagents were procured from Sigma-Aldrich Chemical Co., Ltd, Singapore, Singapore: Folin–Ciocalteu reagent, gallic acid monohydrate, Whatman Filter Papers No.1 (WHA1001325) ethanol and the chemicals for natural deep eutectic solvent preparation.

2.2 Preparation and screening the natural deep eutectic solvents

In this research, eight NADES were synthesized through the heating method (Wu et al., 2020). The hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) were combined in the appropriate molar proportions, heated to 90°C, and stirred using a magnetic stirrer (model: C–MAG HS 7, IKA Industrie, Humboldtstraβe, Königswinter, Germany). The process was considered to complete when a transparent and uniform liquid was achieved. The specific chemicals used, their corresponding abbreviations, and the molar ratios employed for prepared natural deep eutectic solvents are detailed in Table 1.

Table 1: The NADES employed in this study

No.	Abbreviation	HBD	HBA	Molar ratio
1	Lac-Ery	Lactic acid	Erythritol	2:1
2	Lac-Cho	Lactic acid	Choline chloride	2:1
3	Lac-Pro	Lactic acid	1,2 – Propanediol	2:1
4	Lac-Gly	Lactic acid	Glycerin	2:1
5	Ci-Cho	Citric acid	Choline chloride	2:1
6	Ci-Pro	Citric acid	1,2 – Propanediol	2:1
7	Ci-Gly	Citric acid	Glycerin	2:1
8	Ci-Ery	Citric acid	Erythritol	2:1

A quantity of 1.25 g of WP was weighed and dispersed in 25 milliliters of the prepared NADES with a water content of 20% (g/g) in 100 milliliter amber glass bottles. These mixtures underwent sonication an ultrasonic bath from Rama (model RS22L, Rama Vietnam Joint Stock Company, Vietnam). This ultrasonic bath had a maximum volume capacity of 22 liters and operated at a frequency of 40kHz, offering a maximum ultrasonic power of 900W and a total power of 1500W. This sonication process was conducted at room temperature (30°C) for a duration of 10 minutes. The mixtures were centrifuged at 4500 rpm for 15 minutes using a DM0412 centrifuge (DLAB Scientific Co., Ltd, China). Control solvents, including a 50% ethanol solution, a 50% acetone solution, and distilled water were also employed. The quantification of phenolics in the extracts was performed.

2.3 One-factor experiments

The extraction of phenolic compounds was conducted using an ultrasonic bath. The process of UAE of WP encompassed several distinct conditions, which included varying liquid-to-solid ratios (LSR) within the range of 10 to 70 ml/g, adjusting ultrasonic power levels in intervals of 150W from 0W to 900W, modulating the water content of the NADES between 10% and 50%, and altering the extraction durations from 5 to 30 minutes. The samples were separated using filter paper, and the quantification of phenolic compounds in the extracted samples was conducted.

2.4 Phenolic quantification

The quantification of total phenolic content in the diluted samples followed the procedure established by Nguyen et al. (Nguyen et al., 2022). For each extract, a volume of 0.25 milliliters was mixed with 4 milliliters of distilled water. 0.25 milliliters of a 10% Folin-Ciocalteu reagent was added. Following a 5-minute incubation period, 0.5 milliliters of 7.5% sodium carbonate was introduced, and the extract samples were stored in the dark at room temperature for one hour. The absorbance of the test extract samples at 765 nm was measured using a UV-vis spectrophotometer (Hach DR/2010, LabWrech, Canada). To determine TPC, a standard curve of gallic acid spanning from 0 to 150 mg/L was utilized as a reference. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried basic (mg GAE/g).

2.5 Statistical analysis

The experiments were conducted in triplicate, and the results were presented as the mean value accompanied by the standard deviation. The collected data were subjected to an analysis of variance (ANOVA) with a significance level set at 5% ($p < 0.05$). Following this, multiple-range tests were performed using Minitab 19 software (Minitab, Inc, USA). Graphical representations were created using Origin Pro software (Origin Lab, Northampton, USA).

3. Result and Discussion

3.1 Evaluating the extraction effectiveness of NADES

The efficiency of UAE employing NADES was evaluated under specific conditions, which included a liquid-to-solid ratio (LSR) of 20 ml/g, a temperature of 30 °C, and a 20% water content, with a retention time of 5 minutes, focusing on the determination of TPC. Eight distinct NADES were synthesized by combining HBDs, primarily citric acid and lactic acid, with HBAs like glycerine, erythritol, choline chloride, and 1,2-propanediol. The obtained TPC values were compared with those achieved using conventional solvents, and the results are depicted in Figure 1a. All NADES exhibited a relatively high TPC extraction performance of approximately 4.87 mg GAE/g, surpassing that of ethanol at 3.58 ± 0.12 mg GAE/g. Among the NADES, Lac-Pro demonstrated the highest efficiency, likely due to its similar polarity to Ci-Ery and the phenolic compounds found in WP (Vo et al., 2023). Therefore, Lac-Pro was selected for further experimentation in this study.

3.2 Effects of the liquid-to-solid ratio

Figure 1b illustrates the impact of LSR on TPC during the utilization of NADES in UAE. As the LSR rose from 10 to 70 ml/g, there was a notable 1.54-fold increase in TPC (refer to Figure 1a). This rise in LSR led to several beneficial effects. Firstly, it enhanced the contact area between Lac-Pro (a component of NADES) and the target compounds in the plant material, in this case, phenolics in the WP (Waste Pomace). It caused a reduction in vicious extraction medium (Zheng et al., 2022). Lower viscosity facilitated the cavitation effect, which is the formation of bubbles during the expansion cycles (Patil et al., 2021).

The cavitation threshold, representing the minimum pressure required for initiating bubble growth in the extraction medium, decreased with increasing LSR. This had the consequence of intensifying the cavitation effect, resulting in more intensified bubble collapses. This, in turn, generated more robust shear forces and greater interfacial turbulence. These processes led to increased fragmentation and the formation of pores on the surface of WP, facilitating the diffusion of phenolic compounds into the Lac-Pro solution (Rashid et al., 2023). The combination of an expanded contact area and an enhanced cavitation effect synergistically enhanced the efficiency of phenolic compound extraction from WP (Vo et al., 2023).

However, it's worth noting that as the LSR continued to rise from 60 to 70 ml/g, the recovery of phenolics decreased to 8.02 ± 2.03 mg GAE/g. This decrease can be attributed to excessive cavitation, which can have a degrading effect on phenolics and flavonoids in the plant material, ultimately reducing the overall extraction efficiency (Kumar et al., 2021).

In a related study by Rubiya Rashid et al. (2023), it was noted that as the LSR was elevated to 30 ml/g during the UAE process with NADES for phenolic compounds extracted from apple pomace, TPC rose to 5.8 mg GAE/g. As the LSR was further increased to 50 ml/g, a declining trend in TPC from apple pomace was observed (Rashid et al., 2023). The optimal LSR for achieving the maximum phenolic extraction efficiency from WP was determined to be 60 ml/g.

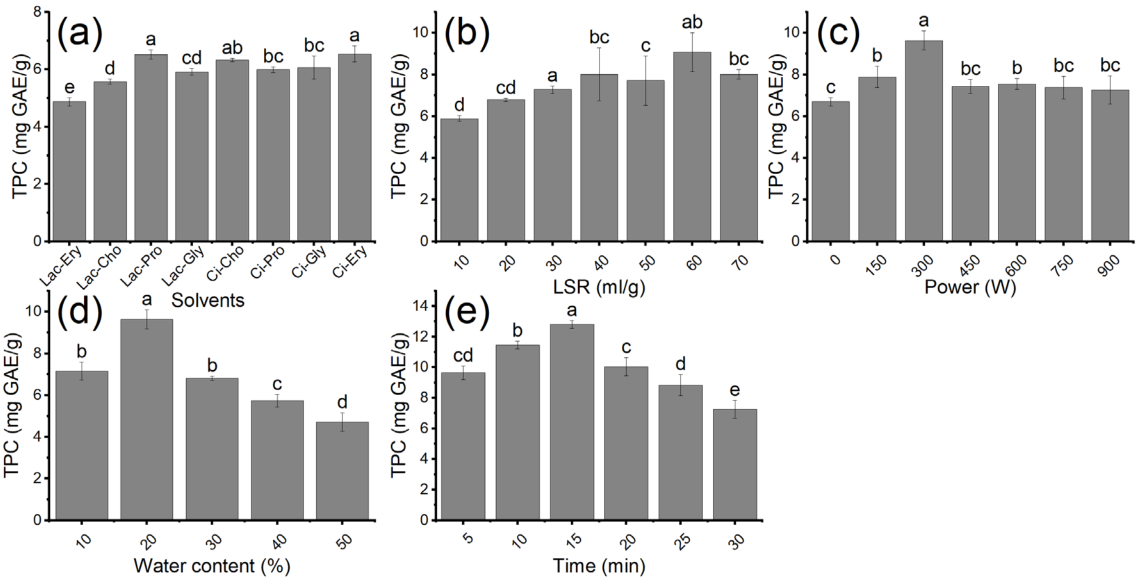

Figure 1: The influence of UAE parameters on TPC: (a) the effect of different solvents; (b) the impact of liquid-to-solid ratio (LSR); (c) the effect of ultrasonic power; (d) the influence of water content; (e) the impact of time different letters show significant statistical differences

3.3 Effect of power

The impact of ultrasonic power levels (ranging from 0 to 900 W) on the extraction yield of phenolic compounds was investigated under specific conditions, including a LSR of 60 mL/g, a temperature of 30 °C, and a 20% water content in Lac-Pro, with an extraction duration of 5 minutes. As depicted in Figure 1c, TPC reached its maximum value when the ultrasonic power was set at 300 W. This heightened extraction yield of phenolic compounds can be attributed to the intensified disruption and sonoporation occurring within plant tissues due to factors such as microshearing, high pressure, and elevated temperature during ultrasonic treatment. These conditions promote enhanced diffusivity and extraction efficiency (Rao et al., 2021). When the ultrasonic power was raised to 900 W, TPC dropped to 7.27 ± 0.68 mg GAE/g. The increase in ultrasonic power leads to an elevated number of cavitation bubbles, resulting in the coalescence, and disruption of these bubbles in non-spherical directions. These combined effects result in a decrease in the strength of collapsing bubbles, which reduces the cavitation effect, resulting in a decrease in the extraction yield (Rao et al., 2021). An ultrasonic power level of 300 W was the optimal choice for achieving a TPC of 9.56 ± 0.16 mg GAE/g from watermelon rind powder when employing NADES-based UAE.

3.4 Effect of the water content in NADES

The impact of varying water content in Lac-Pro within the range of 10% to 50% on TPC was investigated using ultrasonic treatment, with the findings illustrated in Figure 1d. TPC exhibited a 1.35-fold increase as the water content was raised from 10% to 20%. The introduction of water into Lac-Pro resulted in reduced viscosity and density, consequently enhancing the rate of mass transfer from the WP matrix to the extractants. The addition of water to Lac-Pro had the effect of elevating surface tension through an increase in hydrogen bonding. This augmentation in hydrogen bonding acted to reinforce attractive forces, promoting stronger interactions on the surface of NADES (Shafie et al., 2019). The heightened surface tension also encouraged the mutual affinity between Lac-Pro and WP matrix, facilitating the formation of hydrogen bonds between Lac-Pro and phenolic compounds. This occurrence enhanced the solubility of phenolic compounds in Lac-Pro, thereby augmenting the efficiency of the extraction process (Huang et al., 2022).

Conversely, when the water content was increased from 20% to 50%, TPC decreased by a factor of 2.04. The excessive addition of water had the potential to disrupt the hydrogen-bonding networks and create substantial polarity differences between Lac-Pro and phenolics, ultimately diminishing extraction efficiency (Fu et al., 2022). This outcome is consistent with the findings of Zeng et al. (2019), who employed NADES-based UAE to extract phenolics from Chinese wild rice (Zeng et al., 2019). A water content of 20% in Lac-Pro was the appropriate condition for attaining the highest efficiency in the extraction of phenolic compounds from WP.

3.5 Effect of time

The duration of the extraction process can exert a notable impact on both the effectiveness of extraction and the antioxidant properties of the resulting extracts, while also impacting the overall cost of the extraction procedure (Zheng et al., 2022). The kinetic behavior observed for phenolic compounds in WP extracts, as extraction time was varied from 5 to 30 minutes, is illustrated in Figure 1e. Notably, TPC exhibited a substantial increase of 1.33 times when the extraction time ranged from 5 to 15 minutes (Figure 1e). The cavitation effect of ultrasound can enhance various processes within the cellular tissue of the plant matrix, including the creation of pores, hydration, swelling, and fragmentation (Kumar et al., 2021). These phenomena work in conjunction with the pronounced concentration gradient of phenolic compounds between the WP tissues and the extraction solvent, Lac-Pro. These factors enhance the rates of mass transfer and the diffusion of the solvent into the matrix, resulting in increased yields of phenolic compounds (Vo et al., 2023). As the extraction time extended beyond 15 minutes and reached 30 minutes, TPC decreased to 7.27 ± 0.59 mg GAE/g. Extended cavitation effects can potentially result in the degradation of the extracted compounds, diminishing the overall extraction efficiency (Rashid et al., 2023). This observation aligns with the findings of Daniella Pingret et al. (2013), who depicted a similar finding while employing UAE for extracting phenolics from apple pomace (Dey and Rathod, 2013, Xu et al., 2019). An extraction time of 15 minutes was found to be optimal for achieving the highest extraction efficiency of phenolic compounds.

4. Conclusions

In this study, it was determined that Lac-Pro served as the suitable solvent for the extraction of phenolic compounds from watermelon rind powder. The favourable conditions for Ultrasonic-Assisted Extraction using Lac-Pro were identified as follows: a liquid-to-solid ratio of 60 ml/g, a 20% water content, an extraction temperature of 30 °C, and an extraction duration of 15 minutes, resulting in the extraction of 12.81 mg GAE/g of TPC. The findings of this research suggest that the utilization of Lac-Pro-based Ultrasonic-Assisted Extraction represents an environmentally friendly and efficient approach for extracting bioactive compounds from watermelon rind powder.

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