CHEMICAL ENGINEERING TRANSACTIONS
VOL. 108, 2024
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Carlo Pirola, Antonio Espuña

ISBN 979-12-81206-08-3; **ISSN** 2283-9216
DOI: 10.3303/CET24108003

Ultrasonic-assisted Processes to Recover Phenolics and Flavonoids from Passion Fruit Peels

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This study aimed to extract phenolics and flavonoids from passion fruit peels using ultrasonic waves. A combination of ethanol, acetone, and water was used as an ingredient for the solvent mixture, and an augmented simplex centroid design was employed to determine the optimal volume ratio of these solvents to achieve the highest extraction yield. The optimal volume ratio of water, ethanol, and acetone was 0.34:0.38:0.28, respectively, and the total phenolic content and total flavonoid content achieved at 16.50 mg GAE/g db and 10.92 mg RE/g db. Additionally, experiments were conducted to assess the impact of various parameters of ultrasonic-assisted extraction (liquid-to-solid ratio (LSR), ultrasonic power, and temperature) on the recovery yield of phenolics and flavonoids. The suitable conditions for extracting phenolics and flavonoids were LSR of 30 ml/g, an ultrasonic power of 600W, and a temperature of 60°C, with an extraction time of 20 minutes. The total phenolic content and total flavonoid content were at 38.27 mg GAE/g db and 26.02 mg RE/g db. Overall, this research presents an effective approach for obtaining phenolics and flavonoids from passion fruit peels using ultrasonic-assisted extraction.

1. Introduction

Passion fruit, which is predominantly grown in tropical and subtropical regions, is a widely cultivated fruit (Kulkarni et al., 2010). Brazil alone produces approximately 948,100 tons of passion fruit annually, accounting for 50-60% of the world's production (Kulkarni and Vijayanand, 2010). Interestingly, the discarded passion fruit peels constitute a significant portion, ranging from 53-60% of the total fruit mass (Kulkarni and Vijayanand, 2010). Improper disposal of these peels leads to environmental pollution and the need for waste management. However, these peels possess substantial potential as a source of beneficial bioactive compounds such as phenolic acids and flavonoids, known for their ability to counteract free radicals (De Araújo et al., 2021). Free radicals have detrimental effects on essential macromolecules like lipids, proteins, and DNA, contributing to the development of chronic diseases including cancer, respiratory ailments, cardiovascular disorders, neurodegenerative conditions, and digestive disorders (Corrêa et al., 2016). The natural compounds present in passion fruit peels act as scavengers, neutralizing free radicals and interrupting the oxidative chain reactions that harm the body's organs. Therefore, it is crucial to establish an appropriate extraction method to efficiently recover these valuable bioactive compounds from passion fruit peels.

Extraction is the first step in the recovery of bioactive compounds and can be accomplished using various methods, including solvent extraction, mechanical expelling, supercritical extraction, and microwave extraction. However, each of these methods has its limitations. Solvent extraction requires the use of additional solvents, mechanical expelling yields low quantities of compounds, supercritical fluid extraction demands substantial capital investment, and microwave-assisted extraction necessitates the presence of an aqueous phase. (Kumar et al., 2021). Ultrasonic-assisted extraction (UAE) operates by utilizing ultrasonic waves to induce cavitation in the extraction medium (Le et al., 2022; Mathialagan et al., 2017). This cavitation effect arises when the intense rarefaction cycles repel the molecules in the medium, leading to the formation of cavitation bubbles. These bubbles subsequently collapse, causing disruption to the cell walls. The destruction of cell walls facilitates the penetration of solvents into the material, thereby increasing the extraction yield of bioactive compounds (Kumar

Paper Received: 02 August 2023 ; Revised: 13 November 2023 ; Accepted: 27 January 2024
Please cite this article as: Vo P.T., Chieng H.M.N., Nguyen Q.D., 2024, Ultrasonic-assisted Processes to Recover Phenolics and Flavonoids from Passion Fruit Peels, Chemical Engineering Transactions, 108, 13-18 DOI:10.3303/CET24108003

et al., 2021). UAE is considered a sustainable technology due to its ability to achieve high extraction yields while minimizing energy, time, and solvent consumption (Kumar et al., 2021). Given these advantages, UAE has demonstrated its efficacy in enhancing the recovery of bioactive compounds from plants, as observed in the case of (Amiri-Rigi et al., 2016), as well as in the extraction of phenolics from burdock leaves (Moro et al., 2018). . Additionally, the previous research used one solvent for extracting biological compounds from plants, such as extracting phenolics and flavonoids from the stems and leaves of *Grewia carpinifolia* with ethanol, phenolics, flavonoids and tannin from *Ageratum conyzoides* with n-hexane or acetone (Adebiyi et al., 2017; Sultana et al., 2012)... Our study used the solvent mixture to increase the extraction efficiency.

This study aimed to optimize conditions for the extraction of polyphenols and flavonoids from passion fruit peel. Two experiments were carried out with the objective of: (i) optimizing the solvent composition (proportions of water, ethanol and acetone), using simplex centroid design, and (ii) evaluate the influence of ultrasound-assisted extraction parameters on the extraction efficiency of these compounds.

From the way it is written, it is difficult to understand the objective of this research. Reading the results, I suggest: "This study aimed to optimize conditions for the extraction of polyphenols and flavonoids from passion fruit peel. Two experiments were carried out with the objective of: (i) optimizing the solvent composition (proportions of water, ethanol and acetone), using simplex centroid design, and (ii) evaluate the influence of ultrasound-assisted extraction parameters on the extraction efficiency of these compounds."

2. Material and Methods

2.1 Materials

Passion fruit peels were obtained from Nam Viet Company located in Di An, Binh Duong, Vietnam. Subsequently, the peels were dehydrated at a temperature of 45°C until reaching a moisture content of 5%. The dried peels were then subjected to milling to obtain passion fruit peel powder (PFP). The following chemicals and reagents were procured from Sigma-Aldrich Chemical Co.,: 2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent (concentration 1.9–2.1N), gallic acid monohydrate , Whatman Filter Papers No.1 , 6-hydroxy-2,5,7,8 tetramethyl chroman-2-carboxylic acid (Trolox), acetone, iron sulfate heptahydrate, hydrogen peroxide, salicylic acid, sodium carbonate, potassium acetate, aluminum chloride hexahydrate, and ethanol.(Unnecessary description of purity, as the brand of reagents has already been indicated)

2.2 Determination of the optimal proportion of solvents (water, ethanol and acetone) for the extraction of polyphenols and flavonoids.

The study aimed to determine the optimal solvent composition for achieving the highest phenolic and flavonoid recovery yields. Three solvents, namely water (X_2), ethanol (X_1), and acetone (X_3), were used in various volume fractions in the extraction process. The experimental design consisted of a simplex-centroid design with twelve experiments, with each solvent mixture tested at six different levels of volume fractions, while the pure solvent was set at 100%. (unnecessary). Two response variables, total phenolic content (TPC) and total flavonoid content (TFC), were used as indicators to assess the efficiency of different solvent compositions. The significance of the regression coefficients in the special cubic model was evaluated using analysis of variance. Furthermore, triangle graphics were plotted based on the fitted models to visualize the results. The models were validated by conducting experiments at the optimal solvent composition determined from the analysis, ensuring the reliability and accuracy of the findings.

2.3 Evaluation of the use of ultrasound-assisted extraction in phenolics and flavonoids recovery

The extraction of phenolic and flavonoid compounds was performed using an ultrasonic bath Rama (model RS22L, Vietnam), with a maximum volume capacity of 22L and operated at a frequency of 40kHz, with a maximum ultrasonic power of 900W and a total power of 1500W. The ultrasonic-assisted extraction (UAE) of passion fruit peel (PFP) was carried out under different liquid-to-solid ratios (LSR) ranging from 10 to 50 ml/g, ultrasonic power levels ranging from 0W to 900W with intervals of 150W, temperatures ranging from 30°C to 70°C, and extraction times ranging from 5 to 30 minutes. Following the extraction procedure, the samples were separated using filter paper, and the phenolic and flavonoid contents of the extracts were measured.

2.4 Phenolic and flavonoid quantification

The total phenolic content of the diluted samples was assessed using the method developed by Pattrathip Rodsamran and Sothornvit (Rodsamran & Sothornvit, 2019). For each extract, 0.25 ml was mixed with 4 ml of distilled water, followed by the addition of 0.25 ml of 10% Folin-Ciocalteu reagent. After standing for 5 minutes, 0.5 ml of 7.5% sodium carbonate was added, and the extract samples were left in dark conditions for one hour at room temperature. The absorbance of the tested extract samples at 765 nm was measured using a UV-vis

spectrophotometer (Hach DR/2010, LabWrech, Midland, Ontario, Canada). To determine the total phenolic content, a gallic acid standard curve ranging 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 basis (mg GAE/g db).
The determination of total flavonoid content (TFC) was carried out using the Meilin Xu method with a minor modification (Xu et al., 2019). A sample of 0.5 ml was mixed with 1 ml of 96% ethanolic solution, and then 0.1 ml of 1M potassium acetate solution and 10% aluminum trichloride solution were added to the mixture. Subsequently, 4 ml of deionized water was added, and the resulting mixture was placed in a dark environment for 30 minutes. The absorbance of the sample was measured at 415 nm using a UV-vis spectrophotometer. Rutin was employed as a standard for quantification, and the results were expressed as milligrams of rutin equivalent (RE) per gram of dried materials (mg RE/g db).

2.5 Statistical analysis

The experiments were repeated three times, and the results were presented as the mean ± standard deviation. The data were subjected to analysis of variance (ANOVA) with a significance level of 5% ($p < 0.05$). Multiple-range tests were conducted using Minitab 19 software (Minitab, Inc, Pennsylvania, USA). Graphs were created using Origin Pro software (Origin Lab, Northampton, Massachusetts, USA).

3. Result and Discussion

The solvent ratio was determined using simplex centroid design, before the effect of ultrasonic assisted extraction was investigated. The effect of solvent ratio on TPC and TFC was illustrated in Figure 1.

3.1 Optimization of solvent component ratios

The Figure 1 illustrates the effect of solvent on the extraction efficiency of phenolics and flavonoids. The special cubic models for total phenolic content (TPC) and total flavonoid content (TFC) showed high determination coefficients (R^2) of 0.9952 and 0.9943, respectively, which closely matched the adjusted determination coefficients (adjusted R^2), indicating good agreement between predicted and experimental results. The models exhibited significant F-values (103 and 87 for TPC and TFC, respectively), underscoring the significance of the special cubic models (equation (1) – (2)).

$$Y_{TPC} = 3.13X_1 + 9.64X_2 + 5.62X_3 + 12.91X_1X_2 - 10.29X_1X_3 + 25.69X_2X_3 + 199.48X_1X_2X_3 \quad (1)$$

$$Y_{TFC} = 1.70X_1 + 7.13X_2 + 1.65X_3 - 11.74X_1X_2 + 20.91X_2X_3 + 186.57X_1X_2X_3 \quad (2)$$

Water had the most significant effect on the extraction efficiency, followed by acetone and ethanol for phenolics, and ethanol and acetone for flavonoids. The use of pure ethanol and acetone was found to hinder the extraction process by causing protein denaturation and pectin precipitation, limiting the diffusion of solvents into the plant matrix and reducing the yield of phenolics and flavonoids. In contrast, water in the solvent mixture enhanced hydration and improved contact among? between solvents and materials, resulting in increased extraction yields (Vo et al., 2022).

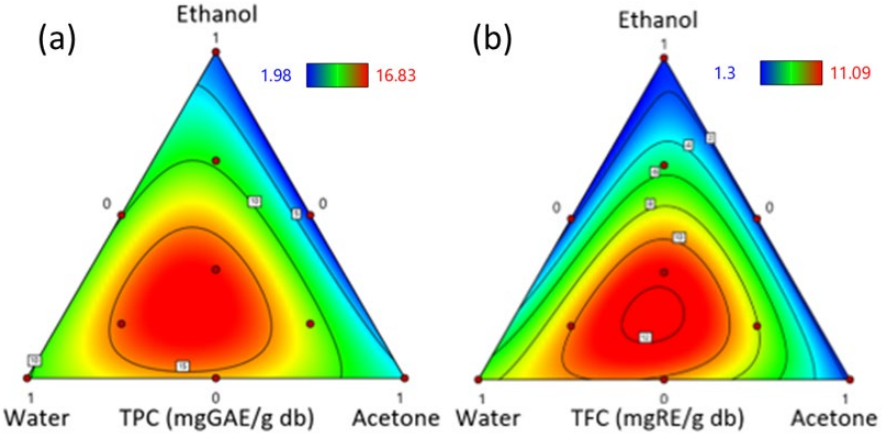

Figure 1: The impact of different solvent combinations on the extraction efficiency of phenolics and flavonoids was investigated in this study.; (a): TPC; (b): TFC

The combination of solvents with different polarities was shown to be effective in recovering phytochemicals, as it improved the solubility of bioactive compounds in the solvent mixture. Similar findings were reported in previous studies involving the extraction of phenolics and flavonoids from other plant sources. Based on the analyses, the optimal solvent volume ratio of ethanol, water, and acetone for the highest recovery of phenolics and flavonoids from PFP was determined to be 0.38:0.34:0.28, respectively, resulting in TPC and TFC values of 16.50 mg GAE/g db and 10.92 mg RE/g db. To validate the reliability of the special cubic models, experiments were conducted at the optimal ratio, and the experimental data for TPC and TFC closely matched the predicted results, with values of 16.89±0.83 mg GAE/g db and 10.11±1.34 mg RE/g db, respectively. In conclusion, the volume ratio of ethanol, water, and acetone at 0.38:0.34:0.28 was determined to be the most suitable for achieving the highest TPC and TFC from PFP. This optimal ratio was consistently used throughout the research experiments.

3.2 Effects of ultrasonic-assisted extraction conditions

The UAE conditions significantly affect the extraction yield of phenolics and flavonoids from PFP; its effects are presented in Figure 2a-h. Figure 2a-b demonstrates the impact of liquid-to-solid ratios (LSR) under fixed conditions (300W ultrasonic power, 30°C, and 10 min). There was an increase in the extraction yield of phenolics and flavonoids when LSR changed from 10 to 30 ml/g. It can be attributed to the enhancement of the cavitation effect, which results from a decrease in the viscosity of the extractant coupled with an increase in LSR (Kumar et al., 2021). The enhanced cavitation effect can impose more sonoporation and fragmentation on the material surface, which improves solvent diffusivity into the plant matrix, thereby elevating the extraction yield of phenolics and flavonoids from PFP. TPC stabilized, while TFC decreased, as LSR continuously rose to 50 ml/g. The excessive LSR can impose more intensity of a cavitation effect on the extraction medium, which can cause flavonoid degradation, decreasing its recovery (Kumar et al., 2021). Moorthy et al. showed a rise in the extraction yield of pectin from pomegranate peel as LSR rose from 10 to 15 ml/g and the continuous growth in LSR to 20 ml/g declined the recovery yield of pectin (Moorthy et al., 2015). Therefore, the proper LSR was 30 ml/g to extract phenolics and flavonoids from PFP.

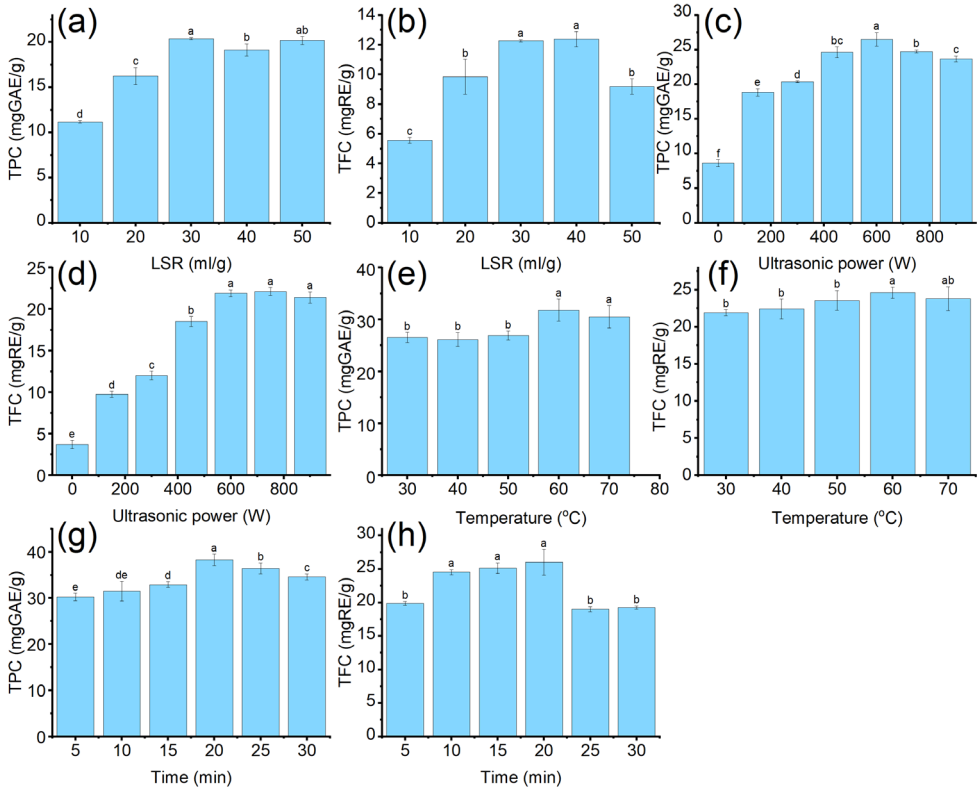

Figure 2: The impact of UAE parameters on TPC and TFC; (a)-(b): the effect of liquid-to-solid ratio (LSR), (c)-(d) the influence of ultrasonic power; (e)-(f) the impact of temperature; (g)-(h) the effect of time.(different letters show significant statistical differences)

The effect of ultrasonic power on the extractability of phenolics and flavonoids from PFP was examined from 0 to 900W at LSR of 30 ml/g, 30°C for 10 min, and the results were illustrated in Figure 2c-d. TPC and TFC increased by 3.1 and 6.0 times, respectively, as the ultrasonic power varied from 0 to 600W. The increase in ultrasonic power can increase the size and intensity of collapsing cavitation bubbles, provoking more sonoporation and fragmentation on plant cell walls and tissues. This phenomenon can increase mass transfer and solvent penetration into plant tissues, accelerating the recovery of TPC and TFC (Kumar et al., 2021). However, with continuous growth in ultrasonic power to 900W, the extraction yield of phenolics decreased, while TFC remained unchanged. The excessive ultrasonic power can modify the structure of phenolics during the extraction process, declining its recovery (Kumar et al., 2021). Therefore, ultrasonic power at 600W was appropriate for extracting phenolics and flavonoids from PFP.

Figure 2e-f showed the effect of temperature (30-70°C) on the recovery of phenolics and flavonoids from PFP at LSR of 30 ml/g, ultrasonic power of 600W, and 10 min. TPC and TFC increased by 1.2 and 1.1 times, respectively, when the temperature rose to 60°C. The increase in TPC and TFC can be attributed to an enhancement of target analyte solubility in the solvent and desorption capacity due to high temperature (Rao et al., 2021). This result was similar to Al-Dhabi et al., who recovered phenolics from the spent coffee ground. In that research, the extraction efficiency of phenolics improved with the rise in temperature from 30 to 45℃ (Al-Dhabi et al., 2017). However, TPC and TFC remained stable as the temperature of the extraction medium increased to 70°C. Therefore, 70°C was suitable for phenolic and flavonoid extraction from PFP.

The impact of time on the extraction efficiency of phenolics and flavonoids was investigated at fixed conditions (LSR of 30 ml/g, ultrasonic power of 600W and 70°C). As presented in Figure 2g-h, the extraction yield of phenolics and flavonoids rose to 20 min, while the continuous extension of extraction time reduced TPC and TFC. Adequate exposure to ultrasound can increase sonoporation and erosion on the plant cell surface, which results from the cavitation effect. This event facilitates the diffusion of phenolics and flavonoids into extractants and the contact of phenolic, flavonoid, and solvent mixture, increasing their extraction yield (Rao et al., 2021). However, the prolonged extraction time can generate inter-bubble collisions and structural damage of phenolics and flavonoids, decreasing extraction yield (Rao et al., 2021). This trend has been presented to recover bioactive polysaccharides from grapefruit peels, banana peels, and rambutan fruit peels (Kumar et al., 2021). Therefore, 20 min of extraction time was appropriate for recovering phenolics and flavonoids from PFP.

4. Conclusions

The optimal solvent volume ratio of ethanol, water, and acetone was determined as 0.38:0.34:0.28 which provided the appropriate polarity for the recovery of phenolics and flavonoids from passion fruit peel. The optimized conditions for UAE to extract phenolics and flavonoids from PFP were identified as follows: a liquid-to-solid ratio (LSR) of 30 ml/g, an ultrasonic power of 600W, and a temperature of 60°C, with an extraction time of 20 minutes. UAE demonstrated its potential as a efficient technique for the extraction of bioactive compounds from PFP, which can become the potential source of bioactive compounds for industrial application.

Acknowledgments

We acknowledge Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for supporting this study.

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