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Influence of ultrasonic treatment parameters on extraction yield of limonin from Wenling Gaocheng peels analysed by HPLC-UV

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Abstract

Ultrasound-assisted extraction (UAE) is a promising technique for isolating bioactive compounds from plant materials, as ultrasound disrupts cell walls through mechanical effects, and facilitates the release of cellular constituents. Despite its growing popularity, the efficiency of UAE for extracting limonin from citrus peels, particularly Wenling Gaocheng, remains understudied. In the present work, we systematically explored the influence of various operational parameters on the yield of limonin using UAE. The limonin content was quantified using a Waters 2695 HPLC system coupled with a Waters 2487 UV detector. The effects of six parameters on the UAE of limonin from Wenling Gaocheng peels was examined through single-factor experiments. We observed that the limonin yield first increased and then decreased with decreasing particle size. The yield also increased as the solvent/material ratio increased, but the rate of increase decreased. Among the tested solvent concentrations, 70% ethanol was found to be the most effective. The yield significantly improved with increasing ultrasonic intensity up to 0.2556 W/cm^2 before decreasing sharply. Between 20 and 70°C, the yield fluctuated slightly, and a gradual improvement was observed with longer extraction times. These findings provided valuable insights for future commercialisation of UAE to extract limonin from citrus peels.

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Introduction

Wenling Gaocheng (*Citrus grandis* × *Citrus sinensis*), a traditional citrus cultivar, thrives in Wenling city, which is situated approximately 300 km away from Shanghai in Zhejiang Province, China. The cultivation of Wenling Gaocheng, as documented in the Chronicle of Taiping County, goes back to at least 457 years ago (Chen and Wang, 2014). In the 1980s, studies revealed that Wenling Gaocheng was an indigenous grapefruit species that existed before first grapefruit emerged in Barbados in 1750 (Wu, 1987). This fruit is celebrated for its distinctive flavour, *i.e.*, moderate sweetness and sourness with mild bitterness, and as a Chinese traditional medicine for its ability to remove internal heat, mitigate alcohol effects, and protect the liver, making it a favoured choice in Zhejiang Province, China (Chen and Wang, 2014). Furthermore, research indicates that dried Wenling Gaocheng samples contain up to 2622.65 ± 96.41 μg/g limonin, and this concentration is significantly greater than that found in other citrus varieties, such as Ponkan (*Citrus poonensis* Hort. ex Tanaka), Huyou (*Citrus paradise* Macf. Changshanhuyou), and navel oranges (*Citrus. sinensis* var. brasiliensis Tanaka) (Sun *et al.*, 2013).

Limonin, a bioactive compound in the limonoid family of triterpenoids, is predominantly found in citrus within families Rutaceae and Meliaceae. Limonin has a wide range of pharmacological activities, including anticancer, antioxidant, anti-inflammatory, antimalarial,

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antiviral, antibacterial, neuroprotective, and bone health-enhancing properties (Fan *et al*., 2019). The pursuit of limonin extraction from plants has intensified, and various methods are being explored. One common extraction technique is Soxhlet extraction (Kaur *et al*., 2023), which is known for its high efficiency. However, its lengthy extraction time is a significant drawback. Another method, supercritical CO_2 extraction (Castillo-Herrera *et al*., 2015), is also known for its efficiency. This method leverages the principle that the solubility of limonin in supercritical CO_2 changes under varying pressures and temperatures. Despite its high efficiency, the supercritical CO_2 method has limited industrial application due to the high cost of extraction equipment, and the complexity of controlling operational conditions. Therefore, it is crucial to identify a method to extract limonin from Wenling Gaocheng peels that is both highly efficient and preserves the compound's biological activity. This method would offer significant advantages, potentially streamlining the production process while ensuring that the extracted limonin retains its valuable pharmacological properties.

In recent years, ultrasound-assisted extraction (UAE) has been recognised as a powerful technique for the extraction and isolation of various active compounds from plant materials (Yusoff *et al*., 2022). This cutting-edge method employs high-speed acceleration, intense vibration, stirring, and ultrasonic cavitation to effectively disrupt plant cell walls. This action facilitates the rapid release of active cellular components into the solvent. The efficiency of UAE surpasses that of traditional solvent extraction methods, primarily since it can enhance mass transfer. This technique not only shortens the extraction time, and reduces solvent use, but also minimises the thermal degradation of sensitive compounds (Kumar *et al*., 2021). Compared to supercritical fluid extraction, UAE offers distinct advantages since operational procedures are simpler, and require less expensive equipment; in addition, mass transfer is enhanced through its mechanical effects. However, despite the numerous benefits associated with UAE, studies focusing on its application for limonin extraction from citrus plants remain limited.

Therefore, the objective of the present work was to assess the influence of various parameters on the extraction of limonin from Wenling Gaocheng peels using UAE. These parameters included the size of the sample particles, the ratio of solvent to material, the solvent concentration, the ultrasonic intensity, the extraction temperature, and the extraction time. Among these factors, ultrasonic intensity is a pivotal factor due to the diversity in the specifications and acoustic parameters of different ultrasonic extraction devices (Martinez-Solano *et al*., 2021). These discrepancies lead to varying ultrasonic intensities across different planes and locations within the cleaning tank, resulting in inconsistent reproducibility among devices. Consequently, accurately determining the ultrasonic intensity within an ultrasonic cleaner is essential for guiding the effective extraction of limonin from Wenling Gaocheng peels. The present work could serve as a valuable reference for future industrialisation of limonin extraction from citrus plants using UAE technology.

Materials and methods

Plant materials

Wenling Gaocheng samples were collected from Wenling (28.84°N, 121.11°E) in December 2017, and were sourced from the Wenling Guoqingtang Gaocheng Farm and the Wenling Wugen Mingguo Specialised Gaocheng Cooperation. Overripe and damaged fruit samples were discarded. The samples were selected and peeled, and the peels were chopped manually by hand. The peels were dried in a heating and drying oven (DHG-9423A, Shanghai Jinghong Laboratory Instrument Co., Ltd., Shanghai, China) at 45°C. The drying was continued until the peels achieved a constant weight, indicating that the desired moisture content of 0.05 on a dry weight basis was reached, at which time the drying process was terminated. The dried Wenling Gaocheng peels were crushed using a plant tissue grinder (FW-135 Tianjin Taisite Instrument Co., Ltd., Tianjin, China). The particles were then sieved to sizes of 0.250 - 0.425, 0.180 - 0.250, 0.150 - 0.180, 0.125 - 0.150, 0.106 - 0.125, and less than 0.106 mm. The samples were then sealed in bags, and stored in a desiccator for subsequent analyses.

Chemicals and reagents

The limonin standard (purity ≥ 90%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from Merck KGaA (Darmstadt, Germany). Alcohol and dichloromethane (analytical grade) were purchased from Sinopharm Chemical Reagent Co.,

Ltd. (Shanghai, China). Ultra-pure water was prepared using a water purification system (SMART-N, Shanghai Canrex Analytic Instrument Co., Ltd., Shanghai, China).

Ultrasound-assisted extraction of limonin

The Wenling Gaocheng peel samples were subjected to UAE using an ultrasonic cleaner (SK2510HP, Shanghai Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China) under a maximum input power of 250 W, and a working frequency of 53 kHz. Each conical flask (250 mL) contained 5.0 g of dehydrated peel sample, and an appropriate volume of ethanol solution at various concentrations. The flasks were fixed 3 cm above the inner tank, bottom of the ultrasonic cleaner, and extraction was performed under different UAE parameters (refer to *Experimental design* below for details) at a frequency of 53 kHz. The temperature of the ultrasonic cleaner remained constant (as listed in Table 1) during the extraction process. Filtration was performed after extraction, and the filtrate was evaporated in a rotary evaporator (RE-52AA, Shanghai YaRong Biochemical Instrument Factory, Shanghai, China) at 45°C until the ethanol was completely evaporated. Three extractions were then performed using 100 mL of dichloromethane in total. The dichloromethane phase was retained, combined, and rotary-evaporated to dryness (45°C). Chromatographic grade acetonitrile was introduced into the flask until a 10-mL volume was reached. Any residue adhering to the flask walls was assessed by filtering through a 0.45-μm hydrophobic membrane prior to analysis by high-performance liquid chromatography (HPLC).

Table 1. Values of selected single-factors.

Level	Factor					
	Particle size (mm)	**Solvent/ material ratio (ml/g)**	**Ethanol concentration (v/v, %)**	**Average ultrasound intensity (W/cm^2)**	**Extraction temperature (°C)**	**Extraction time (min)**
1	0.250 - 0.425	5:1	40	0.0203	20	15
2	0.180 - 0.250	10:1	50	0.0495	30	30
3	0.150 - 0.180	15:1	60	0.0986	40	45
4	0.125 - 0.150	20:1	70	0.1595	50	60
5	0.106 - 0.125	25:1	80	0.2556	60	75
6	< 0.106	30:1	90	0.3840	70	90

Measurement and calculation of ultrasonic intensity

The ultrasonic power levels used in this experiment ranged from 50 to 100% of the maximum input power (250 W), corresponding to 125, 150, 175, 200, 225, and 250 W. The ultrasound intensity was measured at a distance of 3 cm from the bottom of the inner tank (water depth: 10 cm, the probe was placed 7 cm below the water surface) using an ultrasonic intensity metre (E-UEC-200I, Hangzhou Success Ultrasonic Power Technology Co., Ltd., Zhejiang, China), as illustrated in Figure 1. The measurements were recorded at each site indicated in Figure 2, and five ultrasonic intensity values were obtained at 2-second intervals to obtain an average. The average ultrasonic intensity at each power level was determined at the measuring plane, and plotted using MATLAB to generate the average ultrasonic intensities. The resulting 3-D distribution graphs showed that the computed average ultrasonic intensities at the plane 3 cm from the inner bottom of the ultrasonic cleaner were 0.0203, 0.0495, 0.0986, 0.1595, 0.2556, and 0.3840 W/cm^2 for ultrasonic powers of 125, 150, 175, 200, 225, and 250 W, respectively. The details of the 3-D ultrasonic intensity distribution maps obtained at various powers are shown in Figures 3a - 3f.

Experimental design

The present work aimed to evaluate the impact of six relevant factors on the limonin extraction yield from Wenling Gaocheng peels through single-factor tests. Table 1 presents these factors and their respective values. A control group that received no ultrasound application was included for comparison with the ultrasonic intensity factors. Single-factor tests were carried out with the following UAE

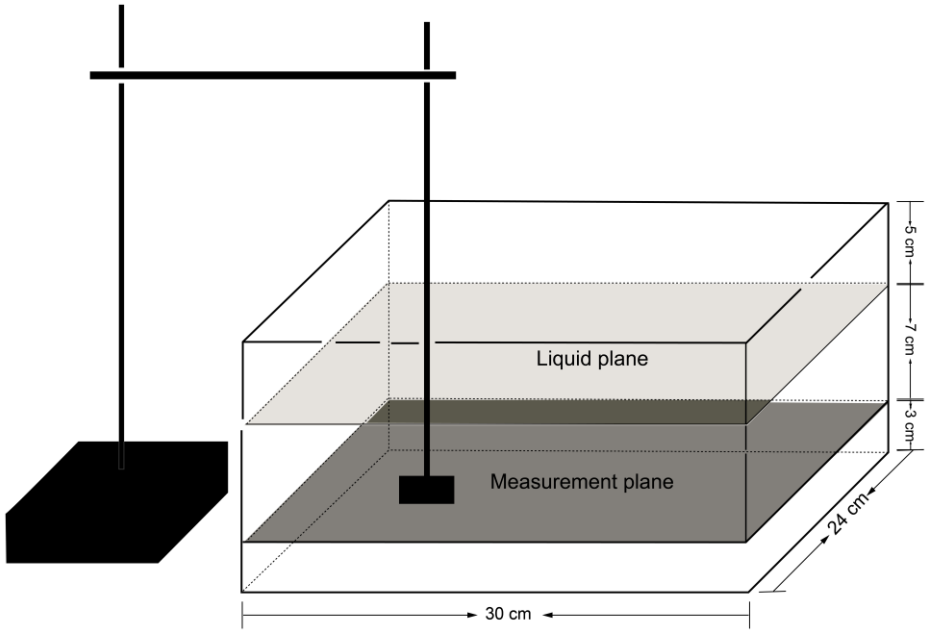

Figure 1. Schematic of ultrasound intensity measurement setup.

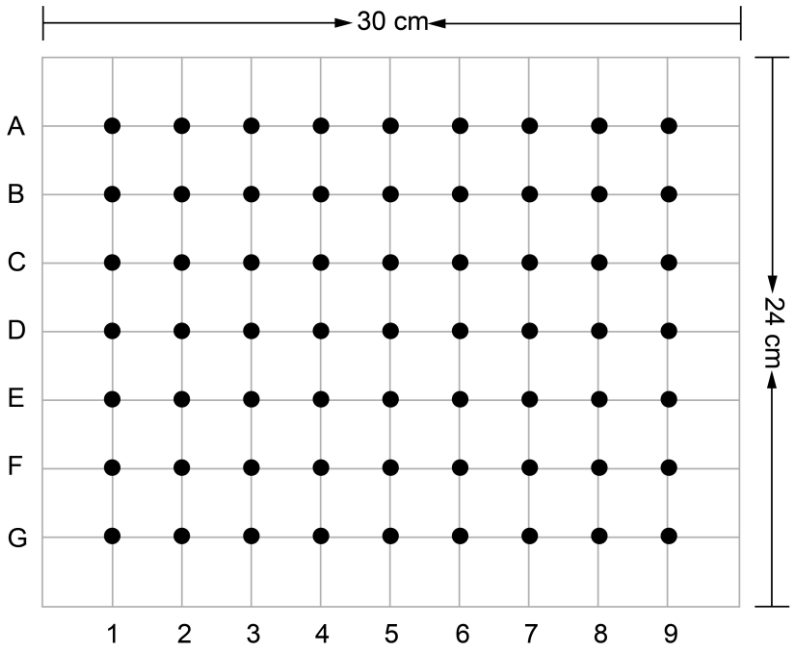

Figure 2. Ultrasound intensity at 3 cm from inner tank bottom of ultrasonic cleaner.

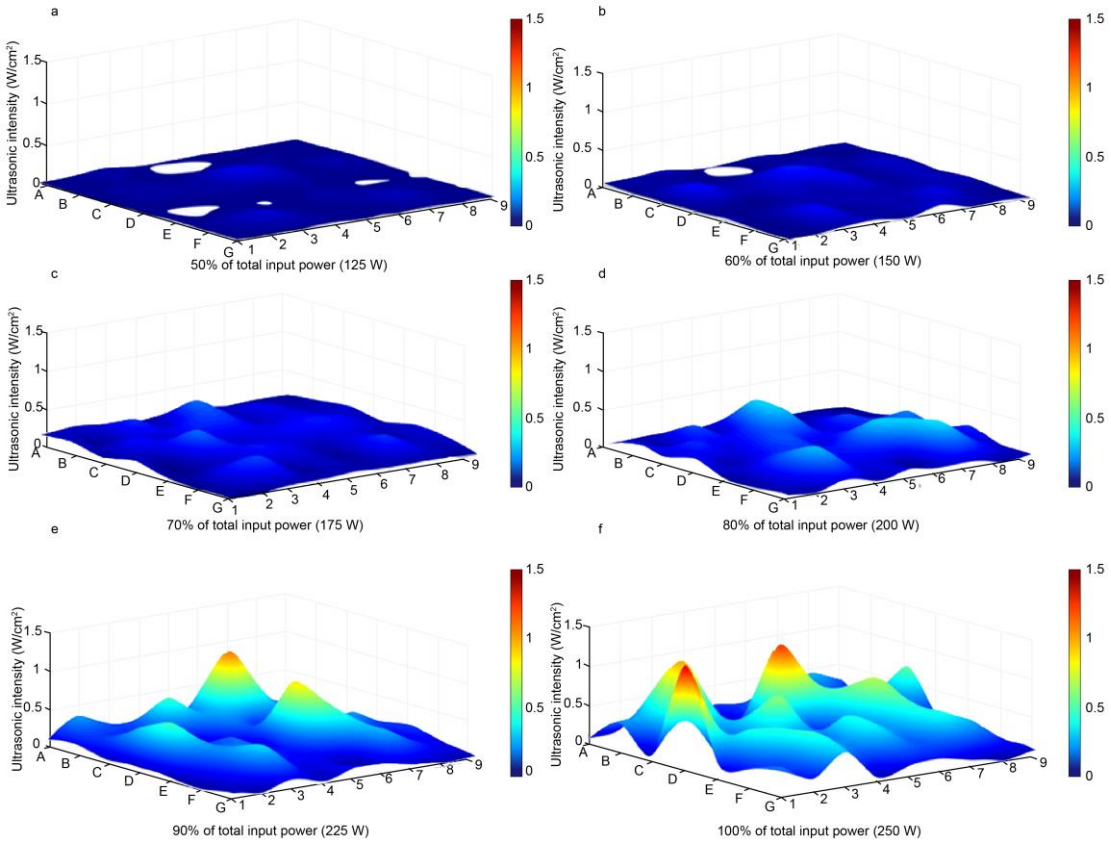

Figure 3. 3-D distribution of ultrasound intensity with measurement plane at **(a)** 125 (0.0203 W/cm^2), **(b)** 150 (0.0495 W/cm^2), **(c)** 175 (0.0986 W/cm^2), **(d)** 200 (0.1595 W/cm^2), **(e)** 225 (0.2556 W/cm^2), and **(f)** 250 W (0.3840 W/cm^2).

parameters: particle size of 0.150 - 0.425 mm, solvent/material ratio of 20 mL/g, ethanol concentration of 70%, ultrasonic intensity of 0.1595 W/cm^2, extraction temperature of 50°C, and extraction time of 60 min.

HPLC analysis of limonin

The limonin content in the extract was determined using a Waters 2695 HPLC system (Waters Corp., Milford, MA, USA) and a Waters 2487 UV detector (Waters Corp., Milford, MA, USA). UV scans of the limonin standard were conducted using a DU800 UV/visible spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA). The analytical conditions for HPLC were established based on the methodology described in prior research (Huang *et al.*, 2019). Preliminary tests were conducted to establish the following conditions: a ZORBAX Eclipse Plus C_{18} column (250 mm × 4.6 mm, 5 μm) as the column, an acetonitrile:water ratio of 45:55 (v/v) as the mobile phase for isocratic elution, a flow rate of 1.0 mL/min, a detection wavelength of 210 nm, a sample volume of 10 μL, and a column temperature of 30°C. A 10 mg limonin standard was weighed accurately using a BS110S analytical balance (Sartorius, Goettingen, Germany). Acetonitrile was added to prepare a 1.0 mg/mL standard solution, which was further diluted to 0.4, 0.3, 0.2, 0.1, 0.05, and 0.025 mg/mL. The limonin standard solutions were analysed using HPLC, and five samples were run in parallel. The peak area (Y) and limonin concentration (X, mg/mL) were correlated using the regression Equation y = 7017548.627x - 7854.429, with R^2 = 0.999992, indicating good linearity in the limonin concentration range of 0.025 - 1.0 mg/mL. The detection limit was 1.793 μg/mL, and the quantification limit was 5.435 μg/mL. The limonin extraction yield (mg/100 g) was calculated using the formula $(C \times V)/M \times 100$, where C = measured limonin concentration (mg/mL), V = standardised volume of the extract (mL), and M = dry weight of the peel sample (g).

Statistical analysis

SPSS 19.0 statistical software was used to analyse the collected data, with differences among data groups being compared using one-way analyses of variance along with Duncan's multiple range test. The results obtained from three parallel samples for each extraction were averaged and presented as mean

± standard deviation. The significance level was set at $\alpha = 0.05$, with differences being deemed statistically significant if $p < 0.05$.

Results

Effect of particle size

Figure 4a shows that the extraction yield of limonin exhibited an inverted U-shaped curve, peaking at a particle size range of 0.180 - 0.250 mm with a maximum yield of 54.43 mg/100 g. In contrast, the yield significantly decreased for particles smaller than 0.106 mm, in which the yield was only 33.26 mg/100 g. This represented a 38.89% reduction from the peak yield, indicating that the particle size significantly influenced the extraction efficiency ($p < 0.05$).

Effect of solvent/material ratio

An increasing solvent/material ratio was associated with a higher limonin extraction yield. As depicted in Figure 4b, a marked increase in yield ($p < 0.05$) was observed within the solvent/material ratio range of 5:1 to 15:1. Beyond this range, the increase in yield was modest from 15:1 to 25 (only 8.12%), suggesting that the yield decreased as the solvent/material ratio increased. The yield was slightly greater at a 30:1 ratio than at 25:1, yet the difference was not statistically significant ($p > 0.05$), indicating that the yield enhancement peaked after the solvent/material ratio was further increased.

Effect of solvent concentration

The yield of limonin increased with increasing ethanol concentration within the 40 - 70% range, as shown in Figure 4c. However, an increase in ethanol concentration from 70 to 90%, particularly at 90%, resulted in a decrease in yield ($p < 0.05$), highlighting the critical role of solvent concentration in optimising the extraction yields.

Effect of ultrasonic intensity

The ultrasonic intensity experiments included a control group without ultrasound application, and a limonin extraction yield of 39.71 mg/100 g was obtained, as shown in Figure 4d. When the average ultrasonic intensity on the measurement plane was increased, a significant increase in the yield of limonin was observed, starting at an intensity of 0.0203 W/cm^2 ($p < 0.05$). Within an intensity range of 0.0203 - 0.15953 W/cm^2, the yields fluctuated; however, these fluctuations did not reach statistical significance ($p > 0.05$). When the ultrasonic power settings (50 - 80%) were adjusted, the ultrasound intensity varied at a plane 3 cm from the bottom of the inner tank of the ultrasonic cleaner, as depicted in Figures 3a - 3d. These variations likely contributed to the observed fluctuations in the yield of limonin. The yield was notably increased to 58.81 mg/100 g when the average ultrasonic intensity was increased to 0.2556 W/cm^2 ($p < 0.05$). Conversely, further increasing the average ultrasonic intensity to 0.3840 W/cm^2 resulted in a significant decrease in yield ($p < 0.05$), highlighting the complex relationship between ultrasonic intensity and extraction efficiency.

Effect of extraction temperature

When the extraction temperature was varied within 20 - 70°C in the ultrasonic cleaner, the yield of limonin did not fluctuate significantly ($p > 0.05$), as depicted in Figure 4e. This result suggested that the extraction yield remained stable within this temperature range.

Effect of extraction time

Figure 4f shows the effect of extraction duration on the limonin yield. No significant difference in yield was observed for extraction times ranging from 15 to 75 min ($p > 0.05$). However, a 90-min extraction significantly enhanced the yield compared to a 15-min UAE ($p < 0.05$), indicating that prolonged extraction could gradually increase the limonin yield from Wenling Gaocheng peels.

Discussion

The present work demonstrated that the optimal extraction yield of 54.43 mg/100 g was obtained when the particle size of the sample was between 0.180 and 0.250 mm. Increasing the solvent-to-material ratio led to a gradual increase in the extraction yield, although the increase progressively decreased. Moreover, selecting an appropriate solvent concentration, especially an appropriate mixture of ethanol and water, was vital for maximising the extraction yield. The use of ultrasound significantly increased the limonin extraction yield; however, its intensity must be controlled within a specific range to prevent limonin degradation. The pH level maintained during the

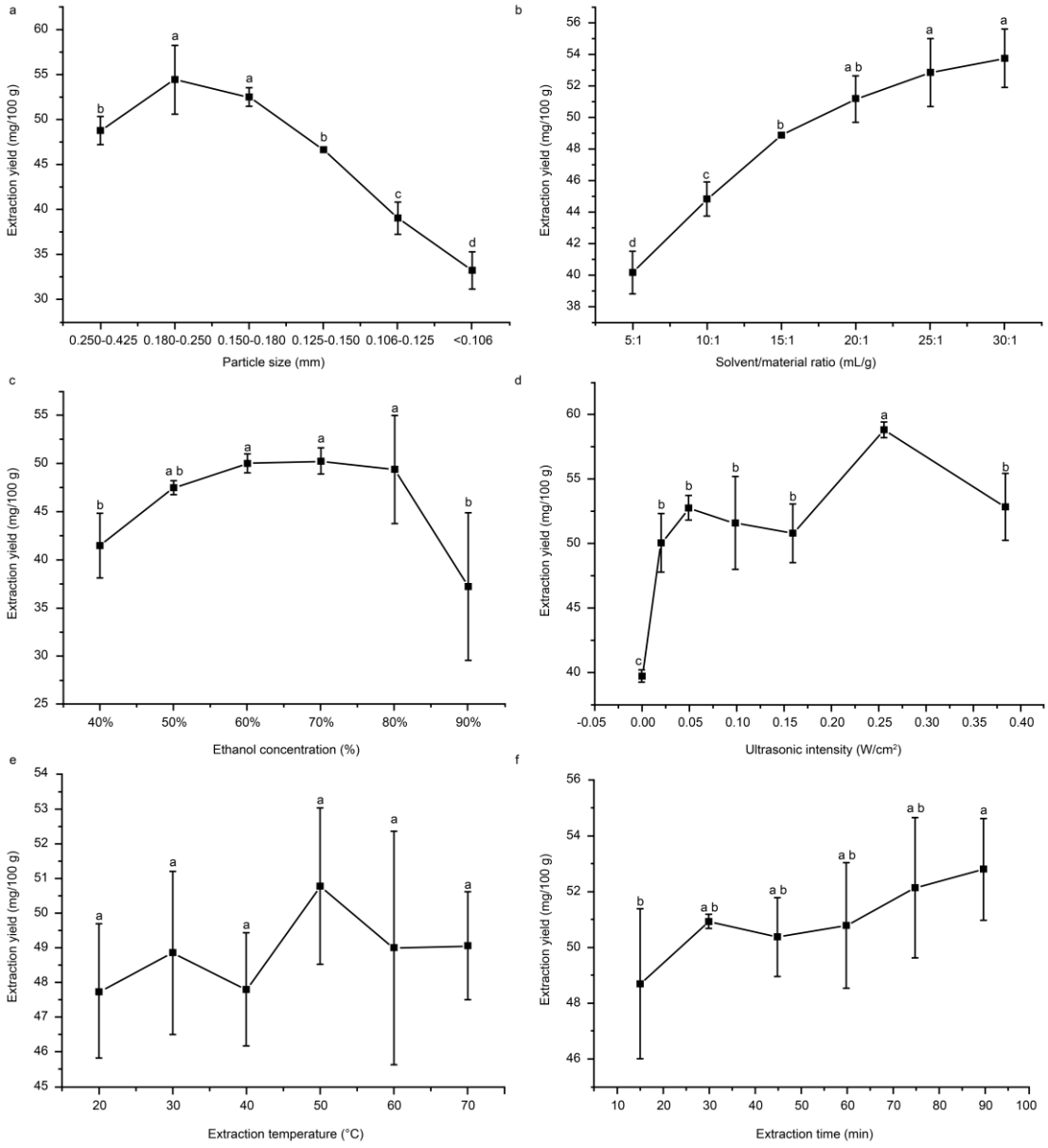

Figure 4. (a) Effect of particle size on limonin extraction yield *via* UAE. **(b)** Effect of solvent/material ratio on limonin extraction yield *via* UAE. **(c)** Effect of solvent concentration on limonin extraction yield *via* UAE. **(d)** Effect of ultrasonic intensity on limonin extraction yield *via* UAE. **(e)** Effect of extraction temperature on limonin extraction yield *via* UAE. **(f)** Effect of extraction time on limonin extraction yield *via* UAE. Different lowercase letters indicate significant differences ($p < 0.05$).

extraction process fell within an optimal range, ensuring the stability of limonin across the temperature conditions examined. However, extending the extraction time did not significantly improve the yield; thus, the time efficiency should be thoroughly assessed. It is crucial to acknowledge that single-factor experiments only reveal the impact of altering one factor on the limonin extraction yield, underscoring the importance of conducting multifactorial analysis to gain a comprehensive understanding of the extraction process.

It is generally believed that the total surface area of a particle and the extraction yield of an analyte are correlated, and this correlation follows the Equation $K = \pi r^2 E$ (Capelo *et al*., 2005), where r is the particle diameter, E is the extraction yield, and K is the constant. Based on this equation, decreasing the particle size would lead to an increase in the extraction yield. However, a deviation from this trend was observed in the present work. The difference observed might have resulted from the properties of the particles with varying sizes used in the present

work. It was difficult to grind the flavedo particles since they were hard after drying, resulting in larger particles that were mainly distributed in the 0.150 - 0.425 mm range. Most of the particles generated from the albedo measurements were smaller than 0.150 mm. This uneven particle distribution has also been observed in prior research (Romdhane and Gourdon, 2002), and occurred due to varying levels of tissue hardness resist crushing. Furthermore, previous research has indicated that the flavedos of Ponkan and pomelo contained greater total limonin and nomilin contents than that of albedos flavedos (Sun, 2006). The results suggested that the relative distribution of flavedo particles was the determining factor for the limonin extraction yield. However, an even distribution of flavedo particles is necessary when the particle size is crucial. Consequently, the extraction yield from smaller particles increased, as a higher yield was generated from particles in the range of 0.180 - 0.250 mm compared to that from 0.250 - 0.425 mm particles (Figure 4a). Due to the difficulty encountered in obtaining particles of different size ranges, we used 0.150 - 0.425 mm particles in all subsequent single-factor tests in the present work.

The phenomenon of the solvent-to-material ratio can be explained as follows (Su, 2012; Dzah and Dzigbor, 2023): for solvent/material ratios ranging from 5:1 to 15:1, the limited amount of solvent used resulted in a higher extract concentration in the solution, which reduced the concentration difference with the interior of the Wenling Gaocheng cell. This reduction does not favour the diffusion of materials. As the solvent/material ratio increased, the difference in the concentration of the Wenling Gaocheng cell interior also increased, accelerating the diffusion of the extract and improving the limonin extraction yield. However, as the liquid-solid ratio increased, mass transfer gradually reached equilibrium, and limonin acquired sufficient space for diffusion. Therefore, the extraction yield increased in smaller increments.

Our findings were consistent with previous research (Phucharoenrak *et al.*, 2022), that examined the effect of solvent concentration on the extraction efficiency of limonin, as an initial increase occurred, followed by a decrease in the extraction rate of limonin as the concentration of the ethanol solution increased. During our study on Wenling Gaocheng peels, we found that after ultrasonic heating and extraction in 40 and 50% ethanol, the sample particles were absorbed and swelled due to excessive extraction-solvent incorporation, causing difficulties in filtration. Additionally, emulsification occurred during the post ethanol extraction phase, leading to a significant reduction in the recovery of limonin extraction and potential depletion of extraction solvent. Similarly, a study on the extraction of pectin from grapefruit rinds reported increased water absorption and tissue swelling under the effects of ultrasound and heat, resulting in a significant increase in pectin recovery (Xu *et al*., 2014). Wenling Gaocheng peels swelled and absorbed water in extraction solvents with high water content. Given the high pectin content in peels, the resulting emulsification led to complications in extraction. This challenge diminished as the ethanol percentage increased since pectin is soluble in water soluble but insoluble in ethanol, consequently improving the extraction yield of limonin. However, beyond a certain point, increasing the ethanol concentration decreased the dissolution capacity of the solution. Pectin began to precipitate and attached to the surfaces of the Wenling Gaocheng particles during diffusion; thus, the ability of limonin to diffuse was diminished, leading to a substantial decrease in the extract yield.

The variation in the extraction rate across the intensity range of 0.0203 to 0.15953 W/cm^2 might have occurred due to the threshold for cavitation was not reached. Limonin extraction was enhanced by ultrasound mainly because the process stirred the solution, and led to mass transfer. We observed a significant increase of 58.81 mg/100 g ($p < 0.05$) in the yield of limonin extraction as the average ultrasonic intensity increased to 0.2556 W/cm^2. This increase occurred because ultrasonic cavitation, which was triggered by exceeding the media's cavitation threshold, effectively broke down the plant cell walls, facilitating the release of cellular content, and improving the limonin extraction yield (Panda and Manickam, 2019). This effect, which is well documented in the literature, aligned with the findings of a previous study (Xie *et al.*, 2008), in which biological phosphorus removal was increased at an ultrasonic intensity of approximately 0.2 W/cm^2 due to the biological impacts of cavitation. Additionally, another study reported a marked increase in sludge activity from an intensity of 0.2 W/cm^2 to a peak activity value related to cavitation at a similar intensity of 0.2 W/cm^2 (Liu *et al.*, 2007). This increase in mass transfer and fluid mixing favourably affected the rate of biological reactions in

the presence of ultrasound. However, as the average ultrasonic intensity increased to 0.3840 W/cm^2, there was a notable decrease in the limonin extraction yield ($p < 0.05$). This decrease could have resulted from the generation of excessive •OH radicals and H_2O_2 in the extraction medium due to intense ultrasonic activity (Merouani *et al*., 2015). Considering the antioxidant properties of limonin (Singh *et al.*, 2022), this process might have reduced its content and extraction rate, a proposition that warrants further experimental investigation.

At an average acoustic intensity of 0.1595 W/cm^2, ultrasound assistance enhanced the extraction of limonin primarily through stirring the solutions, and generating mass transfer. Low-intensity ultrasound promoted the diffusion of the extract in the solvent, ensuring an efficient extraction process without causing the local high temperatures and pressures (5,000 K and 1,000 MPa) typically induced by cavitation. As a result, limonin was protected from high-temperature damage and oxidation. Studies have shown that in aqueous solutions, the degradation of limonin followed a first-order reaction kinetics model (Jitpukdeebodintra *et al*., 2005). Within a system pH range of 5 to 7, the reaction rate constant remained relatively low, and fluctuated only slightly with changes in environmental temperature (45~80°C). The pH of the 70% ethanol system employed in the present work, measured at 6.32, fell precisely within this optimal range, suggesting that temperature variations in this extraction system had a negligible impact on the stability of limonin, and minimally affected its extraction rate. The observed fluctuations in the extraction rate showed no significant differences, indicating that the extraction process was stable.

The impact of extraction time was examined on a measurement plane with an average ultrasound intensity of 0.1595 W/cm^2, which was established to be below the cavitation threshold based on previously mentioned experimental results. Hence, the promotion of limonin extraction in the system solely resulted from the agitation and mass transfer ability of ultrasound, which facilitated the diffusion of limonin in the solvent. The extracted limonin gradually accumulated as the extraction time increased, indicating a degree of time dependence. However, since cavitation did not occur in the system, the increase in extraction yield tended to be gradual.

Conclusion

To summarise, single-factor tests were performed to examine the impact of six different factors on the extraction of limonin from Wenling Gaocheng peels using ultrasound assistance, thereby laying solid groundwork for further refinement of this method. The primary objective of our future work will be based on the results obtained in the present work to explore the interplay of various influencing factors, and to enhance the use of ultrasound-assisted limonin extraction techniques.

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