Study on extraction process and analysis of components in essential oils of Vietnamese orange peel (*Citrus sinensis*) by microwave assisted hydrodistillation extraction

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Abstract. This study attempted the extraction of essential oils (EOs) from orange peel, and optimized the experimental parameters of the microwave-assisted hydrodistillation process. Gas chromatography-Mass spectrometry was often used for the assessment of the chemical content of the natural oils collected. Optimal conditions for the extraction process consisted of the ratio of 1:3 peel/water, microwave power of 300W and duration of 45 minutes. These conditions gave the highest yield (2%). From the gas chromatographic results, five components were identified in the obtained orange peel EOs with Limonene being the major components (98.416%). Based on availability, orange peel is suggested as an economically viable source for limonene production. Obtained results are expected to aid in mitigation of environmental impact caused by agricultural wastes.

1. Introduction

Essential oils (EOs) are mixtures of diversified aromatic compounds that are widely applied in the perfume, food, pharmaceutical, and other industries. Compounds constituting EOs are mainly formed from monoterpenic hydrocarbons, sesquiterpene and their derivatives such as esters, alcohols, and aldehydes [1, 2]. Orange (*Citrus sinensis*) is a member of the genus Citrus and is cultivated primarily in tropical and subtropical climates. The typical tree of *Citrus sinensis* is small with many branches, forming a spherical canopy. Young shoots contain many sharp spines. Orange flower is white-colored, single and grows under leaf axils. Orange fruit skin could be either blue, greenish yellow or fresh orange with many oil veins. It was shown that the volatile content in orange peel was around 85-99% while the
non-volatile components accounted for 1-15% of total content [3]. Essential oils amounts to around 1.5% of the fruit and contains a number of major components including Limonene (>85%), cymene, myrcene, pinene, citral, citronellal, nootkatone, n-decanal, n-nonanal, n-dodecanal and linalyl acetate [4]. These compounds mainly belong to monoterpenes, sesquiterpenes, hydrocarbon class and their oxygen derivatives [5]. It has been recently shown that the abundance of limonene is responsible for potent antibacterial effects [6,7] and fungal inhibitory properties [8] of orange essential oils.

The orange EOs can be obtained by extraction using various techniques, including distillation and solvent extraction. In addition, extraction of EOs could also be performed using novel methods such as supercritical CO\textsubscript{2} extraction, microwave-assisted distillation and ultrasonic distillation [9-13]. While most newer methods are more cost disadvantaged in comparison to conventional extraction techniques, they have been shown to be more efficient in isolating essential oils from plant materials [14,15]. For example, recent works have pointed out that the aid of microwave could contribute to reduced extraction time and environmental impacts and improve the yield and quality of the EOs in hydrodistillation processes [16,17]. Although the use of microwave could be energy-intensive, improvements in yields and product quality could potentially offset the increased energy consumption and higher initial costs by appropriate selection of process parameters. This study aimed to justify the microwave-assisted hydrodistillation in extraction of EOs from orange peels. We examined the impact of several important process parameters including microwave power, material/solvent ratio and time on EOs yield. In addition, the obtained EOs was analyzed using GC-MS to determine its composition [18,22]. The study results are expected to aid in improving existing distillation processes and considering the feasibility of microwave in larger-scale production of essential oils.

2. Materials and Methods

2.1 Materials

Oranges fruits were harvested from Tien Giang province (10°25 13.04 N, 106°17 48.64 E), Vietnam ranging from August to December. Harvested fruits featured a slightly flattened spherical shape, with an average weight of 235.9g. The peel of the ripe fruits was yellowish green, lumpy and 3-5mm thick. Decayed fruits were discarded and the remainder were peeled. The peels were then washed and pureed for use in extraction experiments. To ensure freshness for the experiment, orange peels were stored in a cooler.

2.2 Extraction of Essential oil by microwave-assisted hydrodistillation (MAHD)

Microwave is an electromagnetic wave that propagates at the speed of light. Electromagnetic waves with a frequency range of 300 MHz to 300GHz can generate heat when penetrating and interacting with molecules such as water in materials [23]. In this study, the MW71E microwave oven (produced by SAMSUNG, Vietnam) was connected to a Clevenger type device as shown in Figure 1 to perform the hydrodistillation process. Raw materials and distilled water were added to a 1000mL flask installed in the microwave compartment. Under the microwaves’ action, the water in the plant cells is heated up, causing the internal pressure to increase dramatically, the breakage of tissues containing essential oils. The escaped essential oil was then enticed by the steam to the condensing system or dissolving into water covering the outside of the material. The EOs samples collected were dehydrated with anhydrous sodium sulfate and stored in amber until analysis and preserved vials. Investigated parameters including the ratio of materials/water, the capacity and extraction time were surveyed by one-factor-at-a-time method.
2.3 Gas chromatography-Mass spectrometry (GC-MS) analyses
GC-MS analysis was used for the study of components found in Kaffir lime leaves EOs. Name equipment: GC Agilent 6890 N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. GC-MS was collected under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0μL; injection temperature 250°C; an initial was kept at 50°C for 2 min; an increase to 80°C at 2°C/min; an increase to 150°C at 5°C/min; an increase to 200 °C at 10°C/min; and an increase to 300°C at 20°C/min.

3. Results and Discussion

3.1 The effect of microwave power on extraction yield
Figure 2 showed the variation in oil yield concerning the microwave power. As the power increased from 300W to 700W, the recovery efficiency decreased and the peak oil yield was 2%, achieved at microwave power of 300W. Increased microwave irradiation promotes the turbulence of compound movement and the motion velocity, in turn facilitating the diffusion process. In addition, temperature also denatures and destroys cell membranes thanks to the gas bubbles, making the separation process easier [24]. On the other hand, the temperature that is closer to the boiling point of the solvent may promote the volatility of the solvent, accelerating the contact between the solvent and the material, leading to excess oil in the residues and reducing the recovery efficiency of essential oils [24]. In this investigation the microwave power of 300W seemed to be appropriate irradiation capacity that gave the highest oil yield. The detrimental effect of higher irradiation capacity to oil yield could be explained by the increased diffusion rate and reduced viscosity of the material. Furthermore high capacity may cause unwanted reactions and promote the chemical changes of the ingredients in the material, reducing the quality of the obtained essential oils.

3.2 The effect of material/distilled water ratio
During the extraction process, increased amount of used solvent could improve extraction efficiency. This is due to the greater diffusion capacity of the element into the solvent. However, at a certain threshold, the recovery efficiency will increase insignificantly even though the amount of solvent continues to increase. At the same time, the excess use of solvents is uneconomical and could hinder the solvent separation process after extraction [25]. Therefore, determining the ratio of raw materials/solvents is essential. The results in Figure 3 show that, when the material/solvent ratio was increased, the oil recovery efficiency also increased. At the raw material / solvent ratio of 1: 2, the
efficiency reached 1.6% and increased to 2% when rising the ratio to 1:3. Continuing to increase the ratio of raw materials/solvents from 1:3 to 1:4, to 1: 5 and to 1:6 induced decline in the recovery efficiency of essential oils to 1.8%, 1.4%, and 1.2%, respectively. Therefore, the ratio of raw materials/solvents of 1:3 was selected for the next investigation.

3.3 The effect of extraction time

The extraction time depends on the material, solvent and extraction temperature. Longer extraction time may improve the extraction efficiency. However, the positive relationship only holds to a certain limit of extraction duration where further time prolongation does not increase the extraction efficiency of the EOs [26]. On the other hand, longer extraction time also impairs the quality of essential oils and is more time- and energy- consuming [26]. Figure 4 demonstrated the extraction efficiency at different time levels. It was observed that when the extraction time is increased from 5 minutes to 50 minutes, the oil recovery efficiency also increased. After 45 minutes, after the oil recovery efficiency had reached the peak of 2%, prolonging the time did not seem to improve the yield. Thus, the 45-minute time period provides the highest efficiency for extracting the orange essential oil.

![Figure 2. The effect of power to extract](image1)

![Figure 3. The effect of shell ratio/distilled water](image2)

![Figure 4. The effect of extraction time](image3)
3.4 Chemical composition of orange peel EOs

Table 1 showed the composition of orange peel EOs obtained by GC-MS. Most volatile components in the essential oil had the retention time of around 12 minutes. The main detected compounds were monoterpene hydrocarbons such as Limonene (98.416%), Sabinene (0.071%), β-Myrcene (1.064%), (-)-β-Pinene (0.032%) and 1R-α-Pinene (0.417%). Figure 5 shows the intensity of the peak corresponding to the volatile component in the essential oil. At the retention time of 11.946, the peak with the highest intensity was detected and identified as Limonene, accounting for 98.416% of the content. This result is qualitatively similar to that of Yang et al. (2009) who conducted the extraction of EOs from citrus peels from Jeju Island (Korea), a total of 6 compounds were identified, accounting for 94.5% of the total oil. Accordingly the essential oil compounds were limonene (80.51%), γ-terpinene (6.80%), cymene (4.02%), β-myrcene (1.59%) [27]. In another report, Ferhat et al. (2006) compared the chemical composition, yield and extraction time between MAD and HD methods and showed that Limonene, β-myrcene, linalool, α-sensensal were the primary components in the EOs from orange peel, albeit with varying contents depending on the extraction method. Of which Limonene, a monoterpene hydrocarbon, was the most abundant component with 76.7% and 78.5% content, respectively for MAD and HD [28]. It was articulated that the composition, quality and aroma of pure essential oils may vary depending on growing habitat, environmental factors, the extraction technique, and the plant parts being used [29, 30].

One unfavorable feature of essential oil is the presence of esters, which are often susceptible to hydration and could transform into acid and alcohols when being heated for a long time in water. Therefore, to limit this phenomenon, steam distillation must be performed for a very short time. In this study, essential oils obtained via microwave-assisted distillation contained almost no sesquiterpene, alcohol, phenol and ethylphenol, and aldehydes and was predominated with Limonene. Since limonene has long been found to have remarkable biological activities [31], current results suggest the use of microwave irradiation in production of orange EOs usable for manufacture of antimicrobial, antioxidant, and insecticide agents [32].

Figure 5. GC chromatogram of orange peel oil
Table 1. Chemical compositions of orange peel EOs obtained by MAHD

| Peak | R.T.     | Name             | Molecular formula | Structural formula | CAS number | Pct Total |
|------|----------|------------------|-------------------|-------------------|------------|-----------|
| 1    | 7.261    | 1R-α-Pinene      | C_{10}H_{16}      | H_{3}C-H_{3}C     | 7785-70-8 | 0.417     |
| 2    | 8.997    | Sabinene         | C_{10}H_{16}      | H_{3}C-H_{2}CH_{2}| 3387-41-5 | 0.071     |
| 3    | 9.081    | (-)-β-Pinene     | C_{10}H_{16}      | H_{3}C-H_{3}C     | 127-91-3  | 0.032     |
| 4    | 9.938    | β-Myrcene        | C_{10}H_{16}      | H_{3}C-H_{2}C     | 123-35-3  | 1.064     |
| 5    | 11.946   | Limonene         | C_{10}H_{16}      | H_{2}C-H_{3}C     | 138-86-3  | 98.416    |

4. Conclusions
This research attempted to extract EOs from orange peels using hydrodistillation aided by microwave. The microwave-assisted extraction process provides important advantages concerning vapor diffusion, extraction time and energy consumption. We also determined the influence of important process parameters on the oil yield. Experimental conditions that gave the highest oil yield consisted of the material/water ratio of 1:3, microwave power of 300W, and extraction time of 45 minutes. These conditions corresponded with the optimal recovery of 2%. Five volatile components in essential oils were identified in which Limonene accounted for the highest concentration (98.416%). The major change may be due to a number of factors including geographical location, extraction method and in part the part of the plant used for extraction.

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