Preliminary optimization of extraction process for *Citrus sinensis* Osbeck L. peel essential oil and quality evaluation

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Abstract

The present study performed optimization of the essential oil extraction from *Citrus sinensis* Osbeck L. peel. The conditions of grinding time of 1 min, extraction time of 150 min, and a water-to-material ratio of 1:3 (w/v) on frozen matter yielded 4.20 (%, g/100 g fresh matter) of extract in semi-pilot scaleby hydrodistillation method. *C. sinensis* peel EO was subjected to sensory evaluation, heavy metal contents, physical properties including density, pole rotation angles, and refractive index. D-limonene was identified in the synthesized peel extract with the highest content of 98.12 %. This process may have great potential as an alternative as it allows the recovery of *C. sinensis* by-products and production of high quality essential oils.

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Keywords

Citrus sinensis Osbeck L., essential oil; hydrodistillation; semi-pilot scale, HPLC-MS

1 Introduction

Citrus sinensis Osbeck L. (*Citrus* genus, Rutaceae family) is widely cultivated in Vietnam, especially in South West region. In addition, a large amount of peels are discarded from the juice shops, markets, and processing industry, causing agricultural waste to the environment. However, the orange peel essential oil (EO) and their physiochemical constituents is a valuable material for medicincal purposes. *Citrus* EO accounts for an average of (1-3) % of fresh weight [1]. EO is a complex combination of volatile compounds that has significant applications in the pharmaceutical and food industries [2]. D-Limonene is the main component of *Citrus* EO, with the content varied from 30 % to 99 %, depending on the varieties (e.g. bergamot (30-40) % lemon (40-75) %, and sweet orange (68-98) % [3]. David el al. (1997) reported that D-limonene is a natural monoterpene with profound chemotherapeutic activity and minimal toxicity in preclinical studies [4]. Besides, EO can inhibit some bacteria *(E.coli, P. fluorescens, P. aerugenosa, Klebsilla pneumonia, Proteus spp.)* as well as the fungi *(Candidia albicans)* and show antimicrobial, antioxidant, biological properties, herbal fragrance, and anticancer properties[4-6].

Therefore, the present study performed optimization of EO extraction by hydrodistillation at semi-pilot scale and evaluation of chemical constituents, physical properties, and contents of heavy metal.

2 Materials and methods

2.1 Materials

C. sinensis peel was collected from juice shops located in Bac Ninh Night Market, Thu Duc City in February, 2020. Fresh peel was obtained in the early morning and transported to the study location within 2 hours. They were washed thoroughly with water, peeled, finely chopped, and then ground using a grinder (Sunhouse SHD 5323, Ha Noi, Viet Nam.

2.2 EO extraction

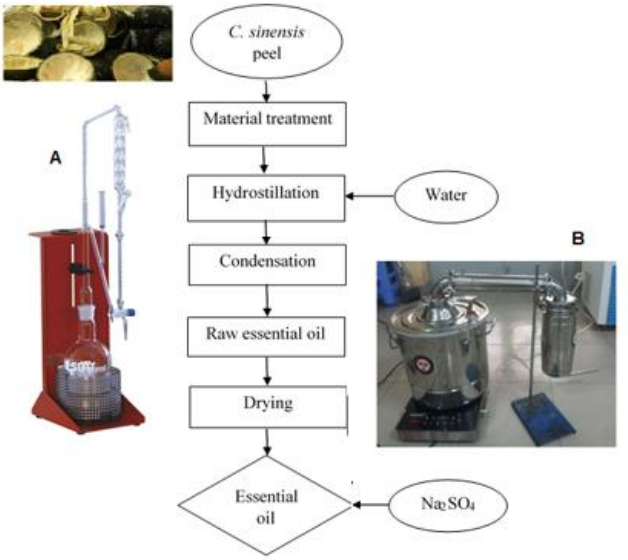

Fig. 1 The sketch diagram of the *C. sinensis* peel EO extraction process. A and B were distillation devices on lab and semi-pilot scales.

C. sinensis peel EO was extracted by hydrodistillation using a distillation device (KOL-2, Behr, Germany, Fig. 1A). The materials were directly immersed in distilled water and heated until the mixture boiled under environmental pressure. This mixture was continued to pass through the cooling system and condenses. The EO was recovered by adding anhydrous salt Na_2SO_4 to the mixture (Fig. 1).

2.3 Preliminary optimizing the extraction of essential oil by hydrodistillation

Using an alternating variable optimization model (Table 1) with the following parameters: The state of matter (X1), time of grinding (X2, min), time of extraction (X3, hour), and ratio material to solvent (X4, w/w). The highest extraction yield (Equation 1) was an important output parameter for collecting optimal conditions. Each experiment was carried out with 100 g of fresh peel. Firstly, the state of raw materials was investigated, including fresh, frozen (−6 ℃, 24 h), dried (70 ℃, 24 h) matter to select the optimal materials $(X1)_{opt}$. Then, the grinding time and extraction time were investigated sequentially, ranging from (0-1.5) min, and (1.5-3) h to select $(X2)_{opt}$ and $(X3)_{opt}$. Finally, the raw material to the solvent ratio (1/1, 1/2; 1/3, and 1/4, w/v) was also surveyed and obtained $(X4)_{opt}$. After that, the obtained yield of *C. sinensis* EO (%, g/100 g fresh matter) was calculated by the following formula:

$$Yield\ of\ EO\ extraction\ (\%) = \frac{mass\ of\ EO\ (g)}{mass\ of\ fresh\ matter\ (g)} \text{ x } 100 \quad \textit{(Eq. 1)}$$

Table 1 Experimental conditions in alternating each variable optimization model

No.	Parameters	X1	X2	X3	X4	Y
1	X1	Fresh-dried-iced matter	1.5	2	1/3	Yield (%, g/100 g fresh matter)
2	X2	$(X1)_{opt}$	0-1.0-1.5	2	1/3	
3	X3	$(X1)_{opt}$	$(X2)_{opt}$	1.5-2.0-2.5-3.0	1/3	
4	X4	$(X1)_{opt}$	$(X2)_{opt}$	$(X3)_{opt}$	1/1-1/2-1/3-1/4	
5	$(Parameter)_{opt}$	$(X1)_{opt}$	$(X2)_{opt}$	$(X3)_{opt}$	$(X4)_{opt}$	

Note: X1: State of matter, X2: Time of grinding (min), X3: Time of extraction (hour), and X4: Ratio material to solvent (w/w). $(X)_{opt}$: Optimized value; Y: Output parameter.

2.4 Production of EO on semi-pilot scale

EO was produced on a semi-pilot scale (Fig. 1B), with a weight of 20 kg of orange peel at the optimal extraction conditions. The EO was evaluated for sensory, physical properties, chemical compositions by high-performance liquid chromatography combined with tandem mass spectrometric (HPLC-MS), and heavy metal contents by atomic absorption spectroscopy (AAS) and atomic emission spectrometry (AES).

2.5 Sensory and physical properties

The *C. sinensis* peel EO was evaluated sensory according to TCVN 11424:2016. The physical properties of EO were identified, including density, pole rotation angles, and refractive index. The analytical instruments used in this experiment included an analytical balance (LS 220A, Precisa, England), an electronic refractometer (ABBE, AR2008), and a polarimeter (Kruss, Germany).

2.6 Analysis of EO components by GC-MS

Gas Chromatography-Mass Spectrometry (GC Agilent 6890 N, MS 5973 inert, Agilent Technologies, Santa Clara, CA, USA) was used to identify the chemical

constituents in *C. sinensis* peel EO. The analytical parameters included 25 µL EO added in 1.0 mL n-hexane and dehydrated with Na_2SO_4; head column (HP5-MS) pressure of 9.3 psi; carrier gas He; flow rate 1.0 mL/min; split 1:50; injection volume 1.0 µL; injection temperature 280 ℃; oven temperature progress included an initial hold at 50 ℃ for 5 min, and increase to 300 ℃ at 10 ℃/min for 5 min.

2.7 Analysis of heavy metal contents in EO

Lead (Pb) and cadmium (Cd) were determined by FAO JECFA Monographs 1 Vol 4, 2006 – Metalic Impurities – (ICP-AES). Arsenic (As) and mercury (Hg) were analyzed by FAO JECFA Monographs 1 Vol 4, 2006 – Metalic Impurities – (Hg-AAS) and FAO JECFA Monographs 1 Vol 4, 2006 – Metalic Impurities – (CV-AAS), respectively. All experiments were performed in Quastest 3 center, HCMC, Viet Nam.

2.8 Statistical analysis

All experiments were performed in triplicates. The results were represented as mean ± standard deviation (S.D.) and analyzed by ANOVA (Analysis of variance) method and all means were compared by Ducan's tests by Microsoft Excel 2016, $p < 0.05$ was considered as statistically significant..

3 Results and discussion

3.1 Preliminary optimization of EO extraction

3.1.1 Influence of state of matter on the EO yield

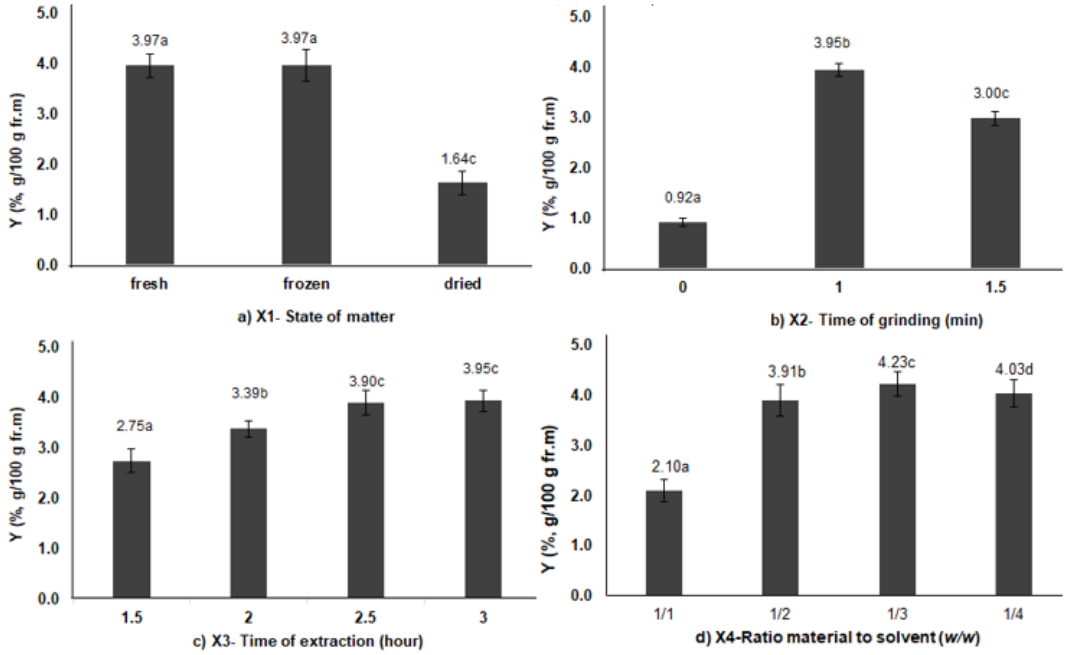

Fig. 2 Effect of several parameters on the yield of EO extraction (%, g/100 g fr.m). (a) State of matter, b) grinding time, (c) extraction time, and (d) ratio of materials and solvent.

Different letters (a-d) showed statistically significant differences between experiments for the same species base on Duncan's test ($p < 0.05$).

The results in Fig. 2a showed the significant difference in EO yields in the fresh, frozen, and dried forms ($p < 0.05$), corresponding to (3.97 ± 0.23; 3.97 ± 0.32 and 1,64 ± 0.24) %, g/100 g fresh matter, respectively. The frozen and fresh forms produced more EO than the dried form and no significant difference in statistics ($p > 0.05$). The lowest yield of EO was obtained from dried material (1.64 %). This result can be explained by the evaporation and loss of EO at high temperature. From there, the frozen form was chosen for subsequent experiments, as this form prolongs the storage time of orange peels during the optimization process. Besides, the microbial growth in orange peels is inhibited at low temperatures to preserve the smell of the EO. Therefore, the frozen matter ($X1_{opt}$) was selected to extract EO from *C. sinensis*.

3.1.2 Influence of grinding time on the EO yield

Fig. 2b showed that the grinding time had a significant influence on the extraction process of EO ($p < 0.05$). The highest yield of EO was found in the samples

obtained after 1 min of grinding (3.95 %), followed by samples in 1.5 min (3.00 %), and the smallest yield in the sample without grinding (0.92 %). This suggested that the peels were crushed, allowing the oil-containing cells to break down and the water to through into the oil-containing vesicles. However, when the size of the material was too small, the EO was lost during the grinding process, thereby reducing the performance. Therefore, the results showed that the optimal grinding time $(X2)_{opt}$ was 1 minute.

3.1.3 Influence of time extraction on the essential oil yield

Fig. 2c shows the effect of extraction time on the yield of *C. sinensis* EO ($p < 0.05$). The extraction efficiency increased significantly from 2.75 % to 3.95 % when the extraction time was prolonged from 1.5 h to 3 h. The highest extraction yield was obtained at 2.5 hours of extraction $(X4)_{opt}$. This can be explained by the initial EO evaporation from the petal surface, thus enabling extraction. After that, the volatile components diffuse from the undestroyed reservoirs in the internal part, and the extraction reaches saturation. From there, the optimal extraction time of 2.5 h was selected to reduce the time and energy costs for the distillation process. This result is also consistent with the study by Sikdar and Ramgopal which found that a distillation time of 2.5 h was optimal for distillation [7].

3.1.4 Effect of material-to-water ratio on the EO yield

In hydrodistillation, the material-to-water ratio is also an important factor of the extraction process as it affects the extraction yield andoperating costs. As shown in Fig. 2d, the highest extraction yield was achieved at a material-to-water ratio of 1/3. Low water content at the ratio of 1/1 and 1/2 (w/v) may not be enough to penetrate through the cell membrane, break the EO compound and dissolved the colloids . However, excessively high water content at the ratio of 1/4 (w/v) could dissolve and emulsify the oil, thus reducing oil yield, and requiring more energy to warm up and boiling and increasing device size [8]. As a result, the 1/3 (w/v) ratio was selected as the optimal ratio $(X4)_{opt}$ in this research.

3.2 EO extraction at semi-pilot scale

From the results of the optimization experiments, the frozen orange peel was collected to enhance the release of EOs and prolong the storage time. The distillation process at semi-pilot scale was carried out with a ratio of 1:3 (w/v) material and water at the extraction temperature of about 100 ℃ for 2.5 hours. 20 kg of fresh raw materials were used to produce 840 g of EO, corresponding to 4.1 % of yield. The obtained EO was evaluated for sensory, physical properties, chemical compositions, and heavy metal contents.

3.2.1 Sensory and physical properties

The EO had a light-yellow color, a sweet and characteristic scent of orange, and a bitter taste (TCVN 8460:2010). The density of *C. sinensis* EO compared to water at 25 ºC was 0.83642 ± 0.00023. The refractive index and pole rotation angle of the EO were (1.3853 ± 0.0014 and 95.97 ± 0.21)º. As a result, *C. sinensis* EO has a relatively low density and refractive index compared to spearmint, peppermint, and Japanese mint EOs [9].

3.2.2 EO analysis by GC-MS

The compositions of *C. sinensis* peel EO by hydrodistillation included 3 components, including D-limonene (98.12 %), α-Pinene (0.6 %), and β-Myrcene (1.28 %) (Fig. 3, Table 2). The result of the present study was also similar to previous studies, yet differences in content remained. According to Sikdar et al. (2016), EO contains only 4 compounds: α-pinene, octanol, β-mycrene, and D-limonene with D-limonene content of 93.14 % [10]. In TCVN 11424:2016, this EO has been reported with 13 chemical components with the content of D-limonene of 93 %. That the ability to extract EO in the study is more optimal than other studies. This study has provided insights for further domestic research on orange EO to exploit more practical applications and protect the environment.

Table 2 Chemical constituents of the extracted essential oil from *C. sinensis* peel via GC-MS

No.	Retention time (min)	Compounds	m/z	Relative content (%)
1	10.848	D-Limonene	136.23	98.12
2	9.706	β-Myrcene	136.23	1.28
3	8.462	α-Pinene	136.23	0.60

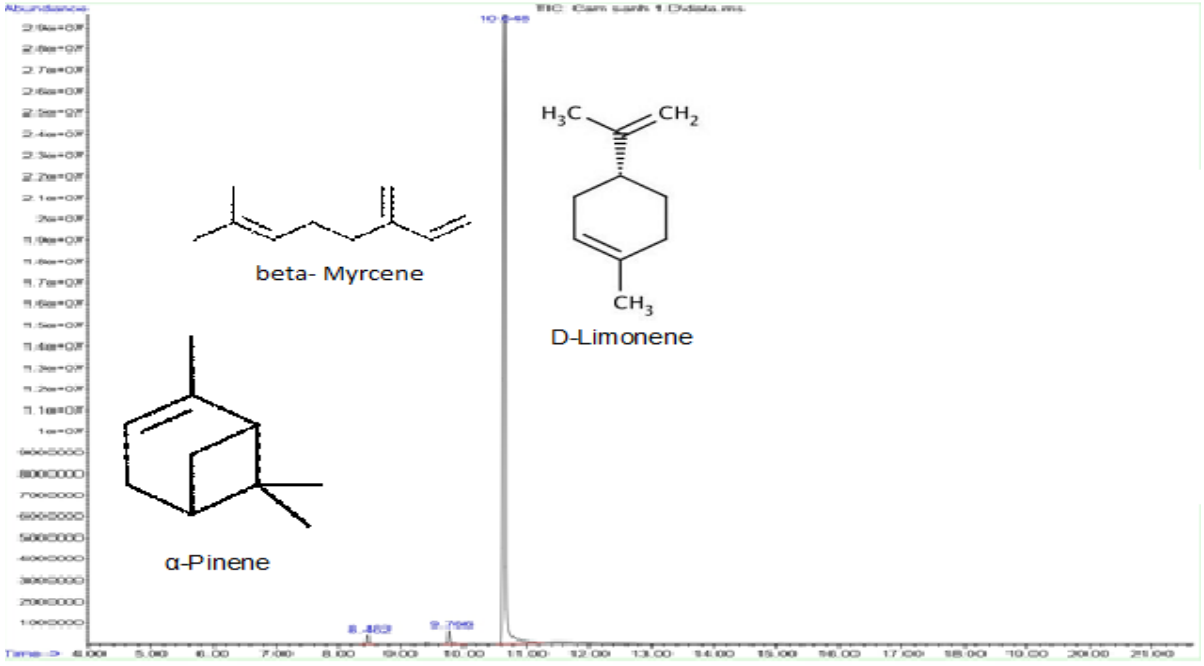

Fig. 3 GC-MS analysis for *C. sinensis* EO

3.2.3 Analysis of heavy metals content in EO

Using some modern analytical techniques, the concentrations of heavy metals in *C. sinensis* EO were qualified. Pb, Cd, and As content did not detect because their contents were below LOD of (0.05, 0.02, and 0.01) mg/kg, respectively. Hg content below 0.07 (mg/kg) is in line with the safety declaration and the product can be commercialized.

4 Conclusion

To recover wastes from *Citrus* processing, a thorough study was carried out on *Citrus sinensis* Osbeck L. peel. The hydrodistillation extraction at semi-pilot scale produced a high yield of EO (4.20 %). The content of D-limonene of the obtained EO was significantly high (98.12 %), as compared to EO extracted from other species. The quality and chemical composition of the EO fulfilled the commercialization standards. This process allows recovery of *C. sinensis* by-products, thereby providing an alternative source of health-promoting nutrients for the industry.

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Tối ưu bước đầu quy trình chưng cất lôi cuốn hơi nước tinh dầu từ vỏ Cam sành (*Citrus sinensis* Osbeck L.) và đánh giá chất lượng tinh dầu

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Tóm tắt Nghiên cứu bước đầu tối ưu quy trình chiết xuất tinh dầu từ vỏ Cam sành (*Citrus sinensis* Osbeck L.) đã được thực hiện trên quy mô semi-pilot với hiệu suất đạt được 4,20 (%, g/100 g mẫu tươi). Điều kiện chưng cất lôi cuốn hơi nước tối ưu gồm có nguyên liệu vỏ Cam đông lạnh, xay nhỏ, tỷ lệ khối lượng nguyên liệu và nước là 1/3, thời gian 150 phút. Tinh dầu Cam được đánh giá các chỉ tiêu cảm quan, hàm lượng kim loại nặng, và các chỉ tiêu vật lý gồm có tỷ trọng, góc quay cực, hệ số khúc xạ phù hợp TCVN. Đặc biệt, kết quả thành phần D-limonene chiếm 98,12 %, cao hơn các tinh dầu Cam thông thường. Điều này cho thấy tiềm năng sản xuất tinh dầu chất lượng cao từ phụ phẩm vỏ Cam sành trong trong công nghiệp.

Từ khóa *Citrus sinensis* Osbeck L., tinh dầu, chưng cất lôi cuốn hơi nước, qui mô semi-pilot, HPLC-MS.