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Investigate factors affecting the extraction of pomelo essential oil (*Citrus grandis*) in laboratory and pilot scale

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Abstract

Pomelo (*Citrus grandis*) fruit contains many bioactive compounds that are good for health, such as polyphenols, carotenoids and vitamins. This study aimed to find suitable conditions for extracting essential oils from pomelo (*Citrus grandis*) by hydrodistillation method in the laboratory and steam distillation in pilot scale. The results obtained showed that the appropriate parameters for laboratory essential oil extraction included: pureed material, water-to-material ratio of 3:1 and extraction temperature at 130°C. When scaling up to 10 kg/batch, additional factors affecting extraction were storage conditions, grinding time, degree of compression, extraction time, capacity and purification method. The highest extraction yield was obtained (66 mL/kg dry matter materials) when raw samples were stored at 4ºC, milled for 30 s, compressed 4 cm and extracted for 90 min with 7 kW power (intermittent operation). The chemical composition of essential oil was identified by GC/MS, in which young pomelo contained 90.084% d-Limonene.

1. Introduction

Pomelo (*Citrus grandis*) belongs to the Ructacea family, and is native to tropical and subtropical regions of Asia, especially growing mainly in India, China, and some parts of Southeast Asia (Liu *et al*., 2012; Talon *et al*., 2020). *Citrus* fruit consists of 60 to 65% peel, 30 to 35% internal tissue, and 10% seed by weight (Lu *et al*., 2023). The increasing consumption of *Citrus* fruits makes the processing industry more and more developed leading to a lot of waste and by-products. Approximately 110-120 million tons of *Citrus* waste is generated worldwide each year, affecting the soil environment and groundwater levels (Mahato *et al*., 2020). The solid residues after processing are mainly peels, membranes and seeds. The main compounds of pomelo peels mainly include water, cellulose, hemicellulose, sugars, essential oils and polyphenols (especially flavonoids, naringin, hesperidin) (Tocmo *et al*., 2020).

Pomelo essential oil (PEO) contains many aromatic compounds (mainly D-Limonene) with a characteristic scent, so it is widely used in the food, cosmetic and pharmaceutical industries (Brah *et al*., 2023). In addition, PEO has a wide range of activities with antioxidant, antimicrobial, and anti-inflammatory properties (Hosni *et al*., 2010). It can also affect blood pressure and heart rate, so it is widely used in aromatherapy to relieve stress (Kawai *et al*., 2020).

Many conventional methods for extracting essential oils include hydrodistillation (HD), cold expression, steam distillation, solvent extraction, maceration, and empyreumatic distillation. Darjazi (2014) conducted a comparison of the composition of pomelo peel essential oil using hydrodistillation and cold pressing, in which the cold pressing method showed the highest content of oxygenated terpenes with 8 aldehydes, 10 alcohols, 4 esters, and 1 ketone (Darjazi, 2014). Essential oil of two varieties of *Citrus maxima*- White pomelo and red pomelo of Bangladesh was extracted and characterized by the hydrodistillation method. The result in GC/MS showed that the three main components for white pomelo were limonene (67.58%), β-linalool (7.53%) and neral (6.61%), and for red pomelo were limonene (73.82%), β-linalool (5.42%) and Neral (4.11%) (Chandra Das *et al*., 2022). Mai *et al*. (2022) investigated chemical compositions and evaluated in vitro antimicrobial activity in essential oil extracted by

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supercritical CO_2 from waste *C. grandis* peel. The results showed that the D-limonene component increased significantly to 95.66% compared with the hydrodistillation extraction (87.60%) (Mai *et al*., 2022). The essential oil extracted by the solvent-free microwave method from pomelo peels was mainly composed of limonene, b-pinene, linalool, a-terpineol, nerolidol and ester compounds, of which 85.6% was limonene (Chen *et al*., 2016).

Recently, many advanced extraction techniques such as supercritical CO_2 extraction, microwave or ultrasonic assisted extraction have been demonstrated in terms of efficiency and product yield (Shen *et al*., 2023). However, these techniques are energy intensive and difficult to scale. On the other hand, steam distillation, with its advantages such as ease of operation and industrial feasibility, remains a popular extraction method for large-scale production of *Citrus* essential oil (Machado *et al*., 2022).

As far as we know, there have been many studies on extracting PEO at the laboratory scale but very few reports evaluating the influence of extraction parameters on the quality of essential oil at pilot scale. Therefore, in this study, the appropriate parameters (material size, water/material ratio, and extraction temperature) for the laboratory-scale extraction of pomelo essential oil were investigated. Then, evaluate the effect of parameters such as sample storage temperature, grinding time, material compression, distillation time and capacity for the 10 kg/batch pilot scale extraction process.

2. Materials and methods

2.1 Materials

Pomelo (*Citrus grandis*) were collected from 3-7 months in Bac Tan Uyen district, Binh Duong province. After that, pomelo peels were separated from the fruit, cut into 2-3 cm pieces, removed damaged peel, and washed 3 times with water. Peels were stored at two temperatures, 4°C and 25°C.

2.2 Pomelo essential oil extraction process on a laboratory scale

The raw material was pre–treated, including removing dirt, damaged parts and cut into small pieces 2 × 2 (cm^2), 1.5 × 1.5 (cm^2), 1 × 1 (cm^2), 0.5 × 0.5 (cm^2) or pureed. Then, 100 grams of fresh peel, along with distilled water, were subjected to hydrodistillation using a Clevenger apparatus for 120 min. Due to the difference in density, pomelo essential oil (PEO) separated out of the aqueous phase and collected oil layer was dried with anhydrous sodium sulfate at room temperature in 5 min. PEO was stored at 5°C for further experiments.

2.3 Pomelo essential oils extraction process in pilot–scale

The distillation process with a batch size of 10 kg was summarized in Figure 1. The design of the distillation apparatus is depicted in Figure 2. Briefly, the raw materials undergo pretreatment similar to that of laboratory scale and then the peels were ground to powder with grinding times of 10, 20, 30, 40 s. The crushed peel was placed in the extractor chamber with the compression varying from 0 to 4 cm. Extraction was carried out at different times (30, 60, 90, 120, 150, 180 min). The extracted essential oil was dried with anhydrous sodium sulfate to ensure all water was removed and then PEO was stored at 5ºC to avoid the evaporation and decomposition.

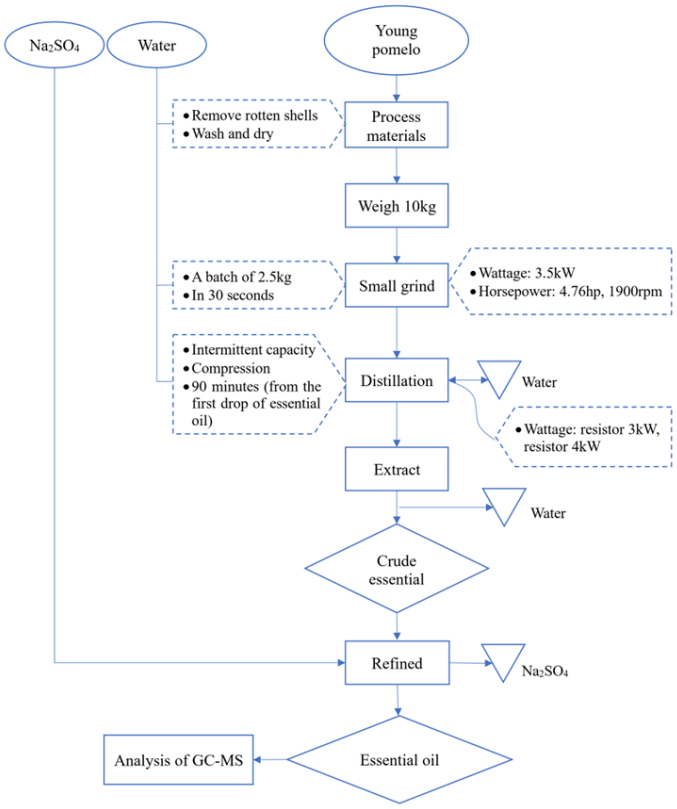

Figure 1. Pomelo essential oil extraction process on pilot scale.

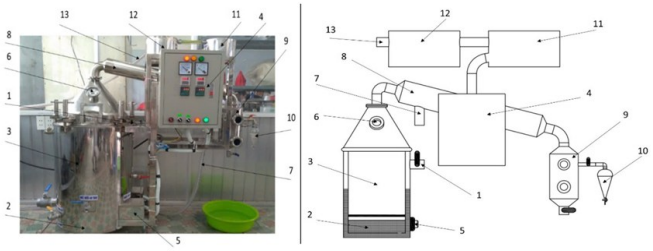

Figure 2. Pilot distillation equipment. 1: Material chamber water supply valve, 2: Material chamber shell, 3: Raw material chamber, 4: Control box, 5: Resistor, 6: Thermometer, 7: Soldering pipe water supply valves, 8: Welding tube, 9: Condenser, 10: Separator funnel, 11: Intermediate average 1, 12: Intermediate average 2, 13: Outward water pipes.

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2.4 Physicochemical properties of raw materials

2.4.1 Moisture content

Raw pomelo peel (5 g) was dried at 105°C to a constant weight, according to the method of Rahman *et al*. (2018).

2.4.2 The content of essential oil

The content of essential oil (mL/kg dry matter) was determined by the ratio of the volume of essential oil to the mass of dry material and was described by the following formula:

$$X = \frac{V.100}{m(100-X)}$$

Where V is the volume of essential oil (mL), m is the mass of the pomelo peel (kg), and X is the moisture content of pomelo peel (%).

2.4.3 Composition of essential oil

Gas chromatography-mass spectrometry (GC/MS) was used to determine the chemical composition of grapefruit essential oil. 30 µL of the essential oil sample was combined with 1.0 mL of n-hexane. The instrument used was a GC Agilent 6890 N with an HP5 compatible MS 5973 inert MS column. A column head pressure of 9.3 psi was selected. The operating parameters of the GC/MS system were as follows: Helium was used as the carrier gas, with a flow rate of 1 mL/min, a division ratio of 1/100, and a pump volume of 1 µL at 270°C. The GC oven temperature program was started with 50°C (2 min hold), then 2°C/min to 80°C, 10°C/min to 200°C and finally 20°C/min (5 min hold) to 300°C.

2.5 Statistical analysis

All experiments were carried out 3 times. The data were collected and evaluated by ANOVA at a 95% confidence interval using the software Statgraphics Centurion Version 20 (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results and discussion

3.1 Research on parameters for the extraction of pomelo essential oils on a laboratory scale

3.1.1 Effect of material size

Samples (73.29% moisture content) with different sizes (2 × 2 cm^2, 1.5 × 1.5 cm^2, 1 × 1 cm^2, 0.5 × 0.5 cm^2 or pureed) were extracted, and the results were summarized in Figure 3. Firstly, ANOVA analysis showed that raw material size significantly affected the obtained essential oil content with 95% confidence. It could be seen that the larger the size, the lower the oil yield obtained because the grinding increased the surface exposed to steam and broke a large amount of oil glands to support the essential oil release (Tran *et al*., 2019; Trujillo-Juárez *et al*., 2021). The extraction yield was significantly improved from 0.8 mL/100 g to 3.1 mL/100 g when replacing materials with a size of 2 × 2 cm^2 with the pureed materials. Therefore, the puree size was selected to investigate the following experiments.

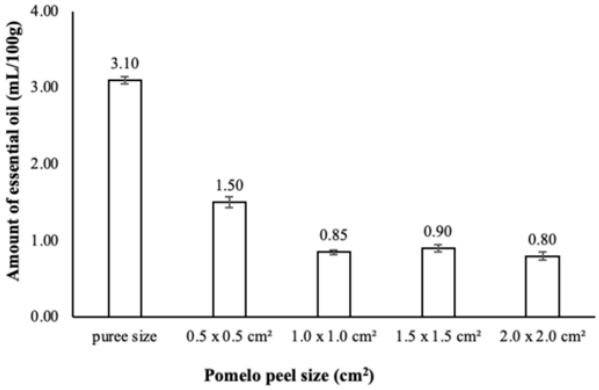

Figure 3. Effect of material size on the extraction of pomelo essential oils.

3.1.2 Effect of water-to-material ratio

When the peels were pureed with water, the water percolated through the cell membranes, causing them to swell. As a result, the oil gland in the shell burst and released the essential oil (Hien *et al*., 2018; Phat *et al*., 2020). Therefore, the ratio between water and raw materials is an important parameter for pomelo essential oil extraction. In the laboratory scale experiment, the water/material ratio was investigated from 2:1 to 5:1 (Figure 4), and as hypothesized, ANOVA analysis showed that the water/material ratio significantly affected the obtained essential oil content with 95% confidence. The amount of essential oil achieved a maximum yield of 3.2 mL/100 g when the water-to-material ratio was 3:1 and tended to decrease as the ratio increased. When the ratio between water and material was small, the colloid on the cell membrane was not fulfilled dissolved, reducing the water vapor permeability rate and the diffusing ability of the essential oil. On the other hand, when the amount of water used

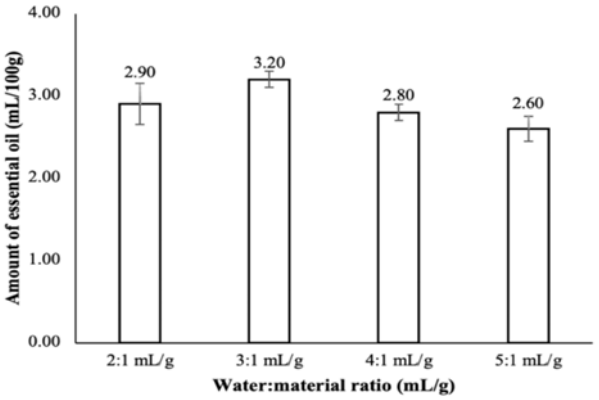

Figure 4. Effect of water:raw material ratio on the extraction of pomelo essential oil.

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was too low, the pressure created was insufficient to completely break down the oil glands and release the essential oils from the mixture. This may explain why the essential oil extraction yield at the 2:1 ratio is lower than the 3:1 ratio. On the contrary, when the water/raw material ratio was too high, the polar components in the essential oil were partly dissolved in the water, causing essential oil loss and reducing extraction efficiency. For that reason, the optimal material and water ratio for further studies was chosen to be 3:1 mL/g.

3.1.3 Effect of steam temperature

The effects of temperatures (120℃, 130℃, 140℃ and 150℃) on the PEO yield extraction were shown in Figure 5. Other conditions, such as material moisture of 73.29%, water/material ratio of 3:1 (mL/g) and distillation time of 120 min, were kept constant throughout the experiment. Firstly, ANOVA analysis illustrated that temperature significantly affected the essential oil content with 95% confidence. The highest essential oil content was achieved at 130℃ (3.32 mL/100 g) while the lowest content was obtained at 120℃. When increasing the temperature from 130 to 150℃, the difference in PEO extraction yield between batches was not significant ($p>0.05$). In general, the Brownian motion of components in the mixture, as well as the diffusivity of essential oils, could be improved when increasing the extraction temperature. Moreover, increasing temperature could denature and destroy the cell membrane of the material due to the formation of air bubbles, making the extraction process easier (Jha and Sit, 2022). Therefore, the amount of essential oil obtained will change with temperature. However, if the temperature was too high, the contact time between the pomelo peels and the steam could be significantly reduced, and therefore the essential oil could not be fully extracted and still remained in the peel pulp, reducing the essential oil content.

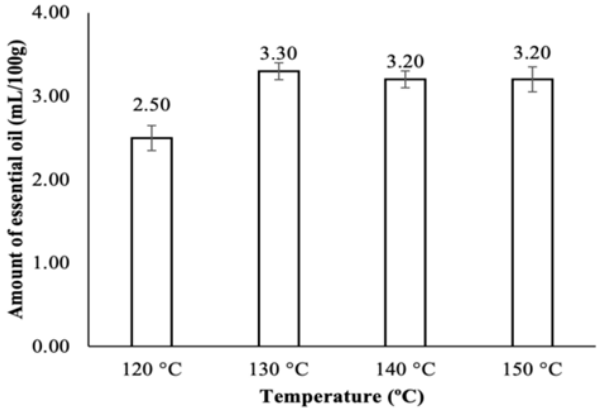

Figure 5. Effect of temperature on the extraction process of pomelo essential oil.

3.2 Investigate the factors affecting the extraction of pomelo essential oil on a pilot scale

Pomelo essential oil extraction was carried out on a pilot scale (10 kg/batch) based on optimized laboratory-scale parameters: pureed raw materials, the water-to-material ratio of 3:1 and the extraction temperature of 130℃. However, scaling up also requires further identification of factors related to raw materials and equipment operations.

3.2.1 Material storage time

Storage time is quite necessary because pomelo is easily damaged, rotten, and the essential oil content will decrease if not stored properly (Tran *et al*., 2019; Huynh Ngo *et al*., 2022). As a result, in this study, the influence of temperature as well as storage time of raw materials on the content of PEO stored at two temperatures of 25℃ and 4℃ from 1 to 4 days was investigated (Figure 6). When stored at room temperature after 1 day, the peel showed signs of wilting, and the amount of essential oil decreased compared to samples used immediately (Hosni *et al*., 2010). Storage time is one of many factors that change the content of essential oils; indeed, Tuan *et al*. (2019) reported that the hydrodistillation yield of PEO stored at room temperature for one day was significantly reduced. In addition, at cold temperatures of 4℃, the amount of essential oil tends to increase and begins to decrease slightly on the 4th day. When young pomelo peels are cooled and then distilled, the oil glands easily break, helping to collect more essential oils, but on the 4th day, the materials begin to dry, and the essential oils are also lost due to evaporation. Therefore, the peel should be kept refrigerated and used in the first 1-3 days to help reduce essential oil loss. In another study, *Citrus aurantium* peels were stored at temperatures of 20℃ and 5℃ to achieve the highest essential oil content (Njoroge *et al*., 2003). Research results showed that weight loss gradually increases during storage, and there were differences between storage times. Storage at room temperature results in more mass loss than cool storage (Hosni *et al*., 2010; Tuan *et al*., 2019). The process of extracting pomelo peel essential oil gave the highest

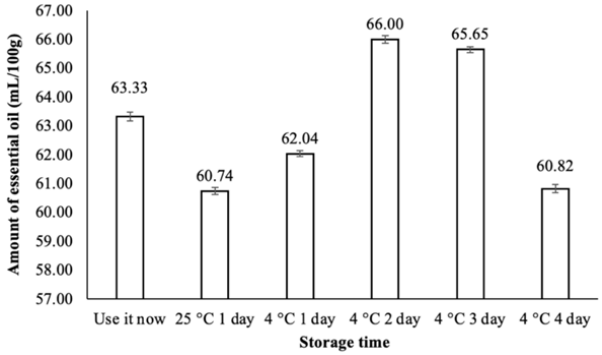

Figure 6. The efficiency of essential oil distillation according to storage time.

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essential oil content when the peel was stored at 4°C for 2 days.

3.2.2 Grinding time and compression

Figure 7 shows the effect of grinding time and material compression on pomelo essential oil extraction. It could be seen that the amount of extracted PEO tended to increase when prolonging grinding time and applying compression to the materials. These results were also confirmed by 2 way-ANOVA and LSD comparison ($p<0.05$). When the ingredients were ground for 10 s, the PEO extraction yield was low (<50 mL/kg dry material), and the difference between samples was quite small. When increasing the grinding time from 20 to 40 s, the extraction yields gradually increased and could reach 66 mL/kg dry material. Moreover, the compression of material also played an important role in improving the extraction yield. An increase in material compression leads to more significant pressure between the material layers, which facilitates breaking down the cell walls and releasing the essential oils inside (Vinatoru *et al*., 2017). For most samples ground for 20 to 40 s, samples compressed to 4 cm showed the highest extraction yield.

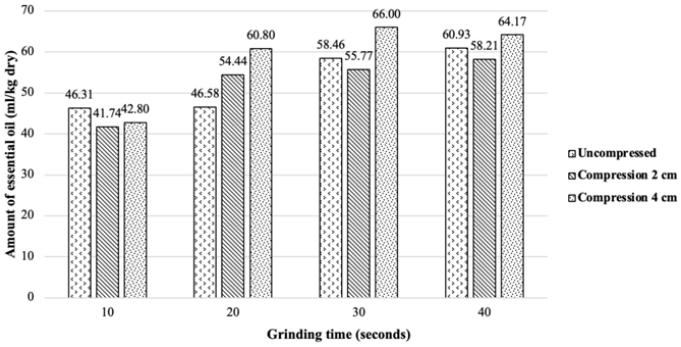

Figure 7. The efficiency of essential oil distillation according to the compression and material size.

3.2.3 Distillation time

Figure 8 presents the change in essential oil content according to distillation time (from 30 to 180 min). In general, the essential oil mainly increased rapidly in the first stage (0 - 60 min) and could achieve a stable yield around 90 min. The optimized distillation time in our study was significantly shorter than the findings in previous studies by Kim Ngan *et al*. (2020) of PEO extraction (115 min) due to the difference between laboratory and pilot equipment. Indeed, prolonging distillation time not only consumed higher energy costs but also affected the quality of the products because the raw materials could burn and lose the natural smell of the essential oil.

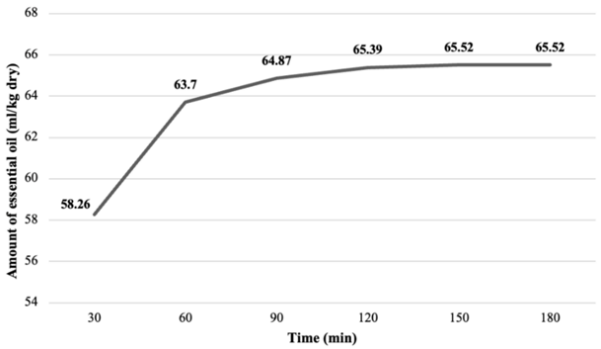

Figure 8. Efficiency of essential oil distillation over time.

3.2.4 Capacity

Figure 9 presents the effect of the capacity machine on the amount of essential oil. When increasing the capacity, the concentration of essential oil and the quantity of water also increased. However, the amount of water required for the extraction process was much higher than the amount of essential oil obtained. It could be seen that a large amount of water was evaporated by heat, but the amount of essential oil obtained did not increase significantly. This could waste heat, and using high capacity will cause the components in the essential oil to easily decompose and change properties, affecting product quality In case the capacity was not large enough, the steam was not enough to absorb all the essential oils in the material. Compared to the study of Masango (2005), the steam distillation device tested three streams with different steam capacities of 20 mL/ min, 5 mL/min and 2.5 mL/min to extract essential oil from wormwood. As a result, a steam flow of 20 mL/min gave the lowest essential oil content, and a steam flow of 2.5 mL gave the highest essential oil concentration, so a lower steam flow gave the highest essential oil content (Masango, 2005). In contrast to the above results, in our study, higher capacity resulted in higher essential oil content. Based on Figure 10, it can be seen that extracting essential oil with two resistors that operated discontinuously achieved higher oil content compared to using two resistors continuously, but not too much higher. Therefore, the pomelo essential oil extraction process with two operated discontinuous resistors (capacity of 7 kW) was chosen to save expenses.

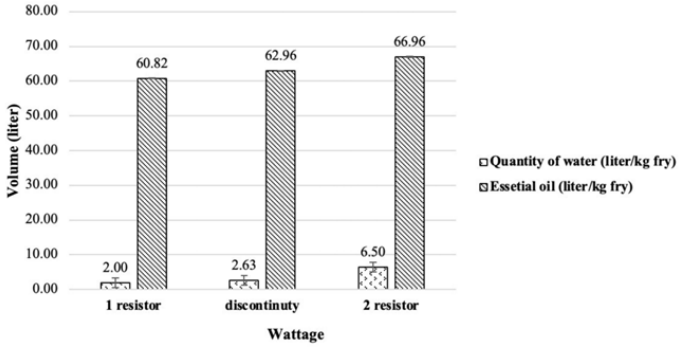

Figure 9. Essential oil distillation efficiency by capacity.

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3.3 Physicochemical composition of pomelo essential oil

3.3.1 Chemical composition of pomelo essential oil

Factors affecting the distillation process of pomelo essential oil on a 10 kg/batch pilot scale equipment include raw materials being pureed in 30 s with a compaction of 4 cm and distilled for 90 min. The obtained essential oil was dehydrated with Na_2SO_4 and evaluated for its chemical composition. The main components of PEO were D-limonene (90.084%), β-myrcene (1.571%), and o-cymene (1.446%). In which D-limonene was the component with the highest concentration, with a signal at a retention time of 12.997 min. Table 1 presents the chemical components and their respective percentages at the pilot scale and laboratory scale. The chemical composition of young pomelo essential oil is mainly isomers of $C_{10}H_{16}$ and $C_{10}H_{18}O$. In this study, the D-limonene content of young pomelo essential oil (90.084%) was higher than that of lemon (*C. lemon* L.) (84.88%), tangerine (*C. reticulata* L.) (82.96%) and lower than pomelo (*C. paradisi* L.) (91.78%) compared to the report of Denkova-Kostova *et al*. (2021). Moreover, the D-limonene content of pomelo essential oil was also quite high when compared to the research of Nguyen Dang Binh *et al*. (2018), who reported an essential oil of 82.54%. When extracted with equipment on a pilot scale, grapefruit essential oil has a very high D-limonene content (>90%). Meanwhile, other compounds are also present in the essential oil composition, such as: β-myrcene, α-phellandrene, o-cymene, γ-terpinene, linalool, cis-limonene oxide, α-terpineol and caryophyllene in smaller amounts.

4. Conclusion

In this study, a pilot-scale extraction technology scheme for young pomelo essential oil was proposed. Pomelo peel was stored at 4℃ to reduce the loss of essential oils, then they were crushed for 30 s, compressed to 4 cm and distilled for 90 min with two operating discontinuous resistors (capacity as 7 kW). Finally purified with Na_2SO_4 to remove the residual water. Young pomelo essential oil is colorless, transparent, shiny, and fragrant with a lot of pungent smell of the peel, a light, cool taste and turns bitter. The main chemical composition of PEO was determined to be D-limonene, accounting for 90.084%. This study also provides a database for further studies regarding optimizing the extraction of essential oils from *Citrus* trees in Vietnam on a pilot scale.

Conflict of interest

The authors declare there is no conflict of interest.

Acknowledgments

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Table 1. Chemical composition of pomelo essential oil.

No.	Retention time (min)	Compounds	Molecular formula	Pomelo essential oils in pilot scale (%)	Pomelo essential oils in laboratory scale (%)
1	7.136	α-Pinene	$C_{10}H_{16}$	-	0.714
2	8.84	Sabinene	$C_{10}H_{16}$	-	0.159
3	8.924	L-β-Pinene	$C_{10}H_{16}$	-	0.205
1	10.908	β-Myrcene	$C_{10}H_{16}$	1.571	0.978
2	11.506	α-Phellandrene	$C_{10}H_{16}$	0.379	0.620
3	12.659	o-Cymene	$C_{10}H_{16}$	1.446	-
4	12.987	D-limonene	$C_{10}H_{16}$	90.084	97.325
5	14.676	γ-Terpinene	$C_{10}H_{16}$	0.542	-
6	17.331	Linalool	$C_{10}H_{18}O$	0.417	-
7	19.108	cis-Limonene oxid	$C_{10}H_{16}O$	0.152	-
8	21.859	α-Terpineol	$C_{10}H_{18}O$	0.707	-
9	23.07	trans-carveol	$C_{10}H_{16}O$	0.271	-
10	24.041	(-)-Carvone	$C_{10}H_{14}O$	0.652	-
11	25.097	2,6- Octadienal	$C_{10}H_{16}O$	0.065	-
12	29.902	Caryophyllene	$C_{15}H_{24}$	0.589	-
		Total (%)		97.528%	100%

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