Application of pressing and hydro distillation technology in the extraction of seedless lemon essential oil (Citrus latifolia Tanaka)

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Abstract. Citrus is one of the most popular tropical fruit tree genera in the world with delicious taste and a rich content of vitamins C, A, fiber, and other essential minerals. The citrus essential oil has the main component being D-limonene, which is a substance with high antibacterial and antioxidant properties, along with various compounds. The experimental process of essential oils extraction from seedless lemon peels by mechanical distillation method combined with hydrodistillation, followed by comparative analysis of the chemical composition of the resulted essential oils. Results have shown that the yield of essential oils obtained from the mechanical pressing and distillation process is 0.089% and 0.10%, respectively. The main compound present in these essential oils is D-limonene with the concentrations in pressed and distilled oils being 58.967% and 38.552%, respectively. The research results show that the technological process of extracting essential oils by mechanical pressing can provide basic understanding about different extraction methods.

1. Introduction

Lemon is an important and abundant tree species belonging to the Citrus genus, Rutaceae family [1]. In particular, the seedless lemon variety (Citrus latifolia Tanaka) originated from California (USA) has been propagated and grown in many parts of the world thanks to the characteristics of the healthytrees, including large leaves, no thorns, bunched fruits, 150-200 kg of annual yield and year-round fruit production. Except for the absence of seeds, the structure of C. latifolia is similar to other citrus fruits, which also composes of green skin termed flavedo and the white skin termed albedo. C. latifolia are often appreciated by consumers not only for their taste but also for their positive health values, which are derived from a good source of vitamin C and minerals [2].

In addition, C. latifolia also contains many bioactive compounds such as fiber, phenolic compounds such as hydroxycinnamic acid and flavonoids; together with essential oils (EOs) which has a significant antibacterial actitvity [3].. Kokate et al (2008) showed that the outermost skin of lemon peel is enriched in EOs (up to 6%), including limonene (90%), citronellal (5%), and some components α-terpineol, geranyl acetate, and linalyl in negligibly small amounts [4]. Research by Kummer et al. (2013) also showed that limonene accounted for 62% of the EOs composition of C. latifolia peel [3]. As one of the most common terpenes in nature, limonene is widely used as a flavoring and preservative agent in daily foods (such as fruit juices, carbonated soft drinks, and ice cream), in the cosmetic and pesticide industries [5]–[7]. Limonene has also been shown to have anti-allergic, digestive, anti-cancer, anticoagulant, insecticidal, antibacterial, and immunomodulatory effects [8]. Therefore, the abundant presence of limonene has made EOs from C. latifolia peels widely applied in food processing, cosmetic, pharmaceutical and daily life [9]–[11].

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Antifungal effect of C. latifolia EOrich in monoterpenes and limonene and demonstrated inhibitory effect on the radial growth of Phasengamularia angolensis which is a common fungal diseases in citrus plants [12]. The antioxidant capacity of lemon EO by DPPH and ABTS scavenging assay exhibited IC50 values of 2.36 mg/mL and 0.26 mg/mL, respectively. This study also demonstrated the protective effect of lemon EO against lipid-induced hyperlipidemia in rats [13]. The results confirmed the positive impact of lemon essential oil on health as well as its potential for use in fungal control and as an antioxidant.

The traditional methods for lemon EO extraction includes direct water distillation, steam distillation, solvent extraction and pressing method. Each method presents different advantages and disadvantages. Therefore, currently, new and modern extraction technologies are being developed to improve the recovery efficiency, as well as the quality of EO. In addition to the use of steam or direct steam distillation, pressing which is a method to obtain EO from fruit peels due to the relative thermal instability of the aldehyde compounds has been reused [14]. This method has the advantage of little or no heat generation. . For a more comprehensive understanding about the efficiency of traditional and modern methods for producing EO, the present study has employed pressing method to recover and evaluate the chemical constituents of the EO obtained from C. latifolia peel.

2. Materials and methods

2.1. Plant materials

C. latifolia was harvested from a garden in Hau Giang province (Vietnam). Lemons are selected on the basis of shape, color, size, uniformity or fungal infection.

2.2. Technological process of producing essential oils by pressing method

Technological process of producing essential oils by pressing method was presented as figure 1. First, *C. latifolia* were purchased with a weight of 20 kg to remove damaged fruits and washed many times with water to remove some external impurities. Next, the seedless lemon was peeled and removed the inner part, then weighed 3.25 kg of the peel. The lemon peels were placed into the screw press device and squeeze twice to obtain 790 mL of EO. Then, this juice was allowed to settle overnight (12h) at < 10°C. The EO was centrifuged to recover at 6000 rpm for 5 min. Then, the obtained EO was dried with anhydrous Na_2SO_4 salt. The EO was evaluated for their physical properties and chemical composition by using GC-MS method.

Fig. 1. Technological process of producing essential oils by pressing method

2.3. GC-MS Measurement Method

The chemical composition of EO samples was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The instrument used was GC Agilent 6890 N (Agilent Technologies, Santa Clara, CA, USA), MS 5973, HP5-MS column, column head pressure 9.3 psi. The GC-MS is installed under the following conditions: He carrier gas; flow rate 1.0 mL/min; split line 1:100; injection volume 1.0 µL; injection temperature of 250 °C. Keep initial temperature at 50 °C for 2 min, oven temperature rise to 80 °C at 2 °C/min, from 80°C to 150 °C at 5°C/min, from 150 °C to 200 °C at 10°C/min, from 200 °C to 300 °C at 20 °C/min and maintained at 300°C for 5 min. Before determining the chemical composition, the essential oil sample needs to be pretreated. 25 µL of essential oil was diluted in 1.0 ml of n-hexane and dehydrated with Na_2SO_4 salt. Filter the sample thoroughly before adding it to the analyzer.

3. Results and discussion

3.1. Physicochemical composition of essential oils obtained by mechanical pressing

The obtained EO has dark green color similar aroma as fresh lemon peel, with a density of 0.884 (g/mL) and content of 0.089%.Chemical composition of EO from *C. latifolia* by mechanical pressing was determined by GC-MS method. The results are presented as shown in Table 1 and Figure 2. Table 1 shows that the obtained EO consists of 20 consituents, including D-Limonene (58.967%), γ-Terpinene (13.201%), β-Pinene (9.595%) as the main compounds, in addition There are also some compounds such as α-Pinene (1,869%), β-Bisabolene (2.885%), Citral (1,912%), α-Bergamotene (1,815%), Sabinene (1,713%), β-Myrcene (1,383%). , Nerol acetate (1.232%), β-Citral (1.223%), Caryophyllene (1.205%), Diisooctyl phthalate (0.507%), β-Cymene (0.425%), Nerol (0.419%), α-Thujene (0.416%) , Citraptene (0.41%), α-Terpineol (0.372%), Herniarin (0.261%), Dibutyl phthalate (0.19%) were identified as minor compounds. D-Limonene is used as an adjunct to commonly used anti-inflammatory and anti-infective drugs [15]. It was confirmed to exhibit anti-inflammatory, anti-diabetic, antioxidant, anti-cancer, anti-allergic, anti-vasoconstrictor and anti-stress activities, in addition to positive effects in treatment of peptic ulcer, colitis, asthma and respiratory inflammation [16].

Table 1. Chemical composition of seedless lemon essential oil by mechanical pressing method

R.T (min)	Compounds	(%)	No.	R.T (min)	Compounds	(%)
7.815	α-Thujene	0.416	11	23.976	β-Citral	1.223
8.074	α-Pinene	1.869	12	25.093	Citral	1.912
9.929	Sabinene	1.713	13	28.235	Nerol acetate	1.232
10.039	β-Pinene	9.595	14	29.903	Caryophyllene	1.205
10.902	β-Myrcene	1.383	15	30.363	α-Bergamotene	1.815
12.65	β-Cymene	0.425	16	32.279	β-Bisabolene	2.885
12.964	D-Limonene	58.967	17	36.208	Herniarin	0.261
14.694	γ-Terpinene	13.201	18	38.633	Dibutyl phthalate	0.19
21.857	α-Terpineol	0.372	19	38.865	Citraptene	0.41
23.482	Nerol	0.419	20	42.136	Diisooctyl phthalate	0.507

When comparing the main components in lemon essential oil obtained by pressing method with other studies, different methods also produce D-Limonene, β-Pinene, γ-Terpinene as the main components with a high proportion. Specifically, according to Martínez-Abad et al. (2020) showed that the composition of lemon peel EO obtained by microwave-assisted distillation analyzed by GC-FID included D-limonene (65,082%), β-pinene (14,517%), and γ - terpinene (9,743%) [17]. In addition, according to the study of Paw et al. (2020), EO was extracted by hydrodistillation and analyzed by GC/MS method. A total of 43 compounds were identified in which D-limonen (55.40%) was the major component, neral (10.00%), Verbenol (6.43%) [18]. In 2010, Di Vaio et al. showed that the main component in lemon peel EO was D-limonen, accounting for 72.5-76.4%, followed by β-pinene (11.6-18.7%), terpinene (2.88-8, 26%), α-pinene (1.4-1.5%) and myrcene (0.99-1.12%) [19]. In 2015, Gök et al. performed a comparison of methods of supercritical CO_2 extraction (SFE), cold pressing and steam entrainment distillation. The results showed high content of D-limonene (71.81%, 70.36% and 72.48%), γ-terpinene (8.72%, 8.91% and 8.88%), β- pinene (6.61%, 6.72% and 6.60%), myrcene (1.55%, 1.59% and 1.73%), sabinene (1.21%, 1.48% and 1, 09%), α-pinene (0.98%, 1.27% and 0.98%) and p-cimene (0.87%, 1.23% and 0.87%), respectively, for the SFE method, CP and HD [20].

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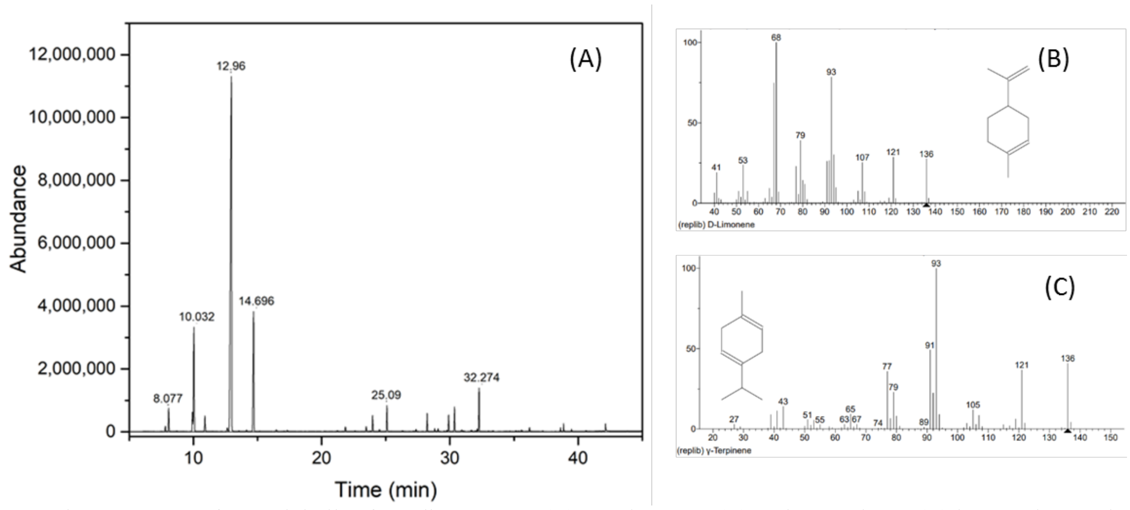

Fig. 2. Chromatogram of essential oils of Seedless Lemon (A), D-Limonene (B) and γ-Terpinene (C) by pressing method

Table 2. Main components of seedless lemon essential oil in some publications

No.	Compounds (%)	This study	A.M.Abad (2020)	Manabi Paw (2020)	A. Gök (2015)	C. Di Vaio (2010)
1	D-Limonene	58.967	65.082	55.40	70.36 - 72.48	72.5 - 76.4
2	γ-Terpinene	13.201	9.743	-	8.72 - 8.91	2.88 - 8.26
3	β-Pinene	9.595	14.517	-	6.60 - 6.72	11.6 - 18.7
4	Citral	1.912	-	10.00	-	-

3.2. The process of direct distillation with water of seedless Lemon residue after the pressing process

C. latifolia pulp after pressing wasthen used for EO extraction through direct distillation with water. The purpose is to evaluate the efficiency of the mechanical pressing process and compare the chemical composition of EO from pressed residues. The pressed raw material (150 ± 0.01 g) was weighed and conducted direct distillation with water for 2 h. The obtained *C. latifolia* peel EO is a transparent liquid with a spicy taste and a very pleasant characteristic aroma, lighter than water, which separates quite clearly in water.

Table 3. Chemical composition of seedless lemon essential oil by hydrodistillation method

No.	R.T (min)	Compounds	(%)	No.	R.T (min)	Compounds	(%)
1	8.078	α-Pinene	0.571	14	25.096	Citral	2.079
2	10.031	β-Pinene	3.565	15	27.365	δ-EIemene	0.309
3	10.905	β-Myrcene	0.72	16	28.238	Nerol acetate	3.03
4	12.644	β-Cymene	1.291	17	28.822	Geranyl acetate	0.968
5	12.923	D-Limonene	38.552	18	29.082	β-Elemene	0.484
6	14.684	γ-Terpinene	9.973	19	29.904	Caryophyllene	2.54
7	16.474	Terpinolene	0.652	20	30.364	α-Bergamotene	3.249
8	17.326	Linalool	2.431	21	32.132	α-Bisabolene	0.384
9	21.236	Terpinen-4-ol	1.806	22	32.282	β-Bisabolene	6.077
10	21.863	α-Terpineol	4.656	23	33.377	Germacrene B	0.377
11	23.508	Nerol	7.709	24	35.161	β-Santalol	0.441
12	23.979	β-Citral	1.585	25	35.365	Allohimachalol	0.403
13	24.532	Geraniol	5.51	26	35.595	α-Bisabolol	0.637

The chemical composition of the analyzed EO sample was identified as D-Limonene (38.552%), γ-Terpinene (9.973%), Nerol (7.709%). β-Bisabolene (6.077%), Geraniol (5.51%), α-Terpineol (4.656%), β-Pinene (3.565%), α-Bergamotene (3.249%), Nerol acetate (3.03%), Caryophyllene (2.54%) , Linalool (2.431%), Citral (2.079%), Terpinen-4-ol (1.806%), β-Citral (1.585%), β-Cymene (1.291%) and Geranyl acetate (0.968%), β-Myrcene (0.72%), Terpinolene (0.652%), α-Bisabolol (0.637%), α-Pinene (0.571%), β-Elemene (0.484%), β-Santalol (0.441%), Allohimachalol (0.403%), α-Bisabolene (0.384%), Germacrene (0.377%), δ-EIemene (0.309%) are compounds with a low proportion. The yield of *C.latifolia* EO obtained by mechanical pressing method (0.089%) and direct distillation method (0.1%) with water from the pulp showed that the condition of the equipment implemented in the study was still low and less effective compared with other methods. In addition, the content of D-limonene which is the main component of *Citrus* EO was also compared between the pressing and distillation process and showedhigh difference (more than 20%). This showed that the content of the main compounds of *C.latifolia* in the mechanical pressing method less degraded than the hydrodistillation method.

4. Conclusions

Results from the present study showed that the extraction process of essential oil by pressing was significantly lower than hydrodistillation methods (0.089%). The *C. latifolia* EO obtained from the pressing method has a more characteristic aroma and darker color than the hydrodistillation method. The chemical composition of *C. latifolia* EOis mainly terpenic hydrocarbons with the main active ingredient being D-Limonene (58,967%), besides γ-Terpinene, β-Pinene and Citral componentswhich were found to be relatively lower than other regions. In addition, there is a large difference in the chemical composition of *C. latifolia* EOobtained from the pressing and distillation processes. These results help to promote the development of a number of food sciences and cosmetic chemistry by utilizing the abundant source of natural compounds and materials.

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