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Original Article

New Elaborated Technique for Isolation and Purification of Limonene from Orange Oil

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Abstract

Essential oil of the orange peel which was extracted by hydrodistillation method showed 50 different mono- and sesquiterpenes on GC-MS analysis, in which limonene had the highest percentage (92.4%). Due to various important uses of the compound, a method was developed for isolation and purification of limonene. In this method, for extra purification, a fractional distillation on Vigreux column under 100 mm Hg vacuum was used which resulted in 96.7% purity for limonene. Subsequent chromatography by two different packages of silica gel and active charcoal columns resulted in 99.4% and 99.9 % purity of limonene, respectively.

Keyword: Column chromatography; Fractional distillation; Limonene; Orange oil; Vigreux.
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1. Introduction

Limonene is a monocyclic terpene which is widely found in the plant kingdom [1]. It occurs in orange rind, lemon, bergamot, dill, mint, cumin, neroli, anethum, myristica, caraway, thyme, cardamom, coriander and many other plants [1, 2]. It is a skin irritant and sensitizer [3, 4]. Limonene has been used in pharmaceutical, food and other industries [3, 5]. The synthetic limonene is also used as insecticide [1], but the natural form is mostly used in aromatherapy.

Anti-tumor activity of the compound in several animal tumor models and *in vitro* experiments has been reported [6, 7]. Limonene inhibits the development of gastric cancers possibly through increased apoptosis [8]. It has been also used as antimicrobial, antiviral, expectorant, sedative, spasmolytic, and antilithic agent [8].

Several methods have been described for extraction of terpenes from *Citrus* genus [9, 10]. In the present study, we used a method of vacuum fractional distillation with Vigreux column [11], and mixed column chromatography methods for higher purification of limonene.

2. Materials and methods

2.1. Plant material

Peels from sweet oranges (Citrus sinensis L.) from north of Iran was prepared and its essential oil was extracted by water distillation.

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Table 1. Chemical composition of the volatile oil of north Iranian orange peel.

Components	Kovats index	Percentage
α-Pinene	939	0.94
Sabinene	976	0.48
β- Myrcene	994	3.89
Limonene	1031	92.42
Cis-Ocimene	1050	0.01
γ- Terpinene	1062	0.01
Octanol	1070	0.07
α-Terpinolene	1088	0.01
Nonanal	1098	0.02
Linalool	1098	0.63
Limonene oxide	1134 or 1139	0.01
Citronellal	1153	0.02
Terpineol <4>	1177	0.04
α- Terpineol	1189	0.07
Decanal	1204	0.38
Nerol	1228	0.03
Neral (Z-Citral)	1240	0.05
Z-Graniol	1255	0.03
E-Citral (Geranial)	1270	0.04
Undecanal	1306	0.01
Neryl acetate	1365	0.01
Geranyl acetate	1383	0.01
Copaene <α >	1376	0.01
Longipinene	1398	0.01
Elemene	1391	0.01
Dodecanal	1407	0.03
Caryophyllene <Z >	1404	0.01
Total		99.25

2.2.Isolation of the limonene

Isolation and purification of limonene firstly achieved by fractional distillation using Vigreux apparatus [11], which was specifically designed for extraction of limonene at different pressures. The condition of the apparatus regarding temperature and pressure was modified for pure limonene extraction. Fractions were received on a multi branch collector in an ice bath using a shell and tube co-current double pipe condenser and trap. Distillation was carried out under 300, 115, or 100 mm Hg pressure, while the temperature was gradually increased, on which respective fractions were collected to a specific head of the collector. The heat range for the collection was experimentally chosen for limonene (25.0-26.3 ºC).

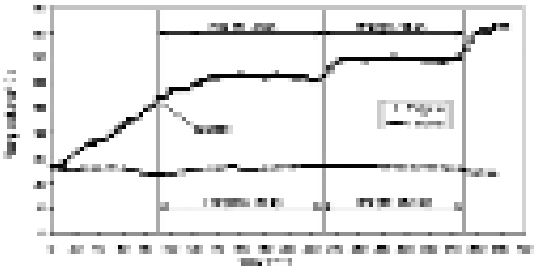

Figure 1: A representation of changes of base and vigreux temperature, showing the more pure limonene obtained in temperature of 23.5-27 C and in higher temperature more impurities are added to the limonene obtained. M: Mantle(base): V.: Vigreux; System Pressure: 300 mmHg.

For further purification of limonene, two columns of silica gel (20-35 mesh) and activated charcoal (packed under gravity and suction) were employed. When the columns were washed with petroleum ether, the compound was obtained with more purity. The purity of limonene was confirmed by TLC [12] followed by gas chromatography-mass spectrometery (GC/MS) analysis.

Table 2. The pressure and temperature range in which fraction 1 was obtained.

System pressure (mmHg)	Base temperature (ºC)	Vigreux temperature (ºC)	Time (min.)[a]	
300	51.0-61.0	23.5-27.0	138	(Figure 1)
300	65.0-75.0	26.0	45	(Figure 2)
115	36.0-46.0	24.0-25.0	96	(Figure 3)
100	34.5-40.0	25.0-26.3	170	(Figure 4)

[a] Legnth of time that fraction 1 was obtained.

2.3. GC-MS spectrometric analysis

The GC-MS analysis of the essential oil of the orange peel and products of fractional distillation and column chromatography were carried out on GC-MS spectrometer and the samples were analyzed under the following operating conditions: Termoquest GC 2000; column DB-5, 30m × 250 µm × 0.25 µm; volume of injection, 0.1 ml; carrier gas: He; flow rate: 1.5 ml/min.; oven temperature: 50-260 ºC with rate of 2.5 ºC/min.; ionization mode: termoquest finnigan mass 2000, electron impact (EI) at 70 eV; transfer line temperature: 265 ºC; ion source temperature: 230 ºC.

The identification of the essential oil and fraction components was performed by the comparison of their relative retention time (RRT) and their mass spectra with those of authentic samples, literature data [13], and computerized MS-data bank (Saturn, version 4). The peak area method was followed for quantitative determination of different constituents; the percentage was calculated relatively [13].

3. Results

GC/MS of the crude extract of the orange oil revealed that 92.4% of the oil was limonene (Table 1). The temperature range in which fraction 1, containing high amounts of limonene, was obtained, by using Vigreux distillation apparatus at different pressures are presented in Table 2 and Figures 1-3.

To obtain high purity, the temperature of the base was gradually increased and the Vigreux temperature was also increased accordingly. Otherwise the results would not be satisfactory (Figure 4). By adjusting the base and Vigreux temperature, plus pressure adjustment, the best temperature and pressure was chosen to obtain higher amounts of limonene. When the base and Vigreux had the shortest distance, a better result was achieved than when they were far from each other (Figures 3, 4). The highest level (96.7%) of purity was observed in 100 mm Hg pressure and when the temperature was between 25-26 ºC on Vigreux (Figure 3).

The above material was then subjected to column chromatography whereby 99.4% and 99.9% purifications were obtained.

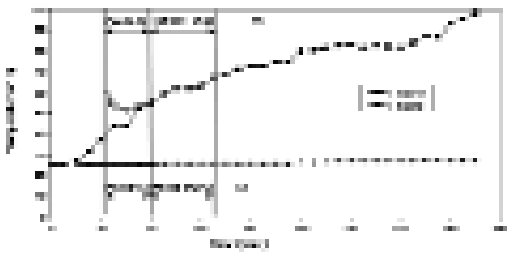

Figure 2: A diagrammatic representation of changes of base and vigreux temperature, showing when the temperature of base was increasing fast. Limonene had the least purities. M: Mantle(base); V.: Vigreux; System pressure: 300 mmHg.

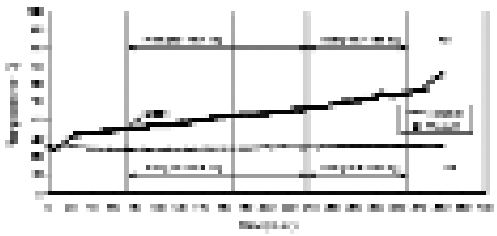

Figure 3: A diagrammatic representation of changes of base and vigreux temperature, applying relative lower pressure, in which pure Limonene was obtained at temperature 24-24.9 ºC. M: Mantle(base); V.: Vigreux; System pressure: 115 mmHg.

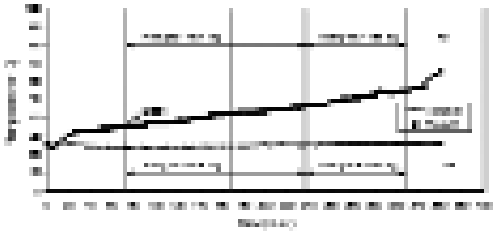

Figure 4: A representation of changes of base and vigreux temperature, showing the closes they get, the purer limonene is obtained. M: Mantle(base): V.: Vigreux; System pressure: 100 mmHg.

4. Discussion

In the present study, three different levels of purity of limonene extracted from orange peel were obtained. In fractional distillation, limonene had the industrial level purity (96.7%), but high purity limonene for aromatherapy (99.4 and 99.9%) was obtained by a column chromatography method. This method can be used in a scaled model for industrial use. Because the industrial use of the terpeneless essential oil of orange peel is of interest, therefore, the preparation of the high purity limonene could be found by fractions 2 and 3. They should be applied in separate columns of silica gel and activated charcoal in which the highest purity of limonene (99.9%) will be achieved.

In conclusion, using fractional distillation under vacuum followed by a column chromatography, limonene at an industrial purity range, and also the highest purity of terpeneless essential oil of orange peel can be obtained.

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