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Evaluation of the quality and composition of lemon (Citrus limon) peel essential oil from an Algerian fruit juice industry

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ABSTRACT/RESUME

Abstract: *Lemon peel essential oil is used in food, medicines, cosmetics and perfumes, detergents, aromatherapy, pathogen inhibition, and insect control. The present work aimed to investigate the physicochemical characteristics and composition of the essential oil extracted from lemon grown in Algeria. The essential oil was obtained by cold-pressing from lemon peels which lemon barks are waste from an Algerian fruit juice industry. The physicochemical properties of the essential oil were determined. Essential oil was also characterised by FTIR-ATR and by GC-MS. The obtained physicochemical properties results revealed that lemon peel essential oil was a pale yellow aromatic liquid which was miscible in ethanol. It had an approximate pH of 6, a specific gravity of 0.894, a refractive index of 1.475 and an acidic value of 2.10 mg KOH/g. The FTIR spectrum revealed the presence of alkanes, alkenes, aromatic compounds and alcohols. A total of thirty-nine chemical compounds were identified based on GC-MS analysis of the lemon peel essential oil with a variation of percentage. These compounds are dividing in three different chemical classes (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes and others). The results showed that the monoterpene hydrocarbons are the most abundant ones in the cold pressed lemon peel essential oil which represent about 93% of the total essential oil. The major constituent was α-limonene (~ 65%) and the main aldehydes were geranial (~ 2%) and neral (~ 1%).*

I. Introduction

Essential oils (EOs) are vegetable products which constituents are a complex mixture of volatile molecules. The EOs can be obtained from different parts of plants by using different extraction methods. Lameaceae, Lauraceae, Myrtaceae and Rutaceae families are particularly rich in essential oils. The qualitative properties and quantitative compositions of EOs are complex. They depend on plant maturity, climatic season, soil type, altitude, storage conditions and extraction method [1-3]. Therefore, each EO has specific properties and thus it is important to be characterised in order to evaluate its quality and then to determine its chemical composition. Lemon (Citrus limon) is a spice of the Citrus. It belongs to the Rutaceae family and it is first originated from Southeast Asia then it is spread to Northeast India, Burma and China [4]. Lemon peels constitute 30–40% of the fruit weight and they are described as waste [5,6]. They are the dominant waste generated by the juice manufacturing industry which companies are currently facing waste management difficulties. Lemon peel essential oil

(LPEO) is an aromatic compound which is commonly isolated from lemon peel by cold pressing. LPEO is widely used in perfume, cosmetic, food and pharmaceutical industries. Numerous papers reported that LPEO have antioxidant [7], antifungal [8] and antimicrobial [2,7], anticancer [9], and anti-inflammatory [10] activities. Lemon EO is generally composed with three main classes: monoterpenes, monoterpene oxygenated derivatives, and sesquiterpenes [11]. Monoterpene hydrocarbons are major components and represent more than 90% of the oil [11,12]. The oxygenated compounds such citral are the major contributor to the flavour and aroma of the oil [11-13]. The antibacterial, antioxidant and anticancer effects are due to its high content of phenolic compounds particularly limonene [5].

In order to evaluate the quality and composition of LPEO, which lemon barks are waste from an Algerian fruit juice industry, the present work investigated physicochemical characteristics and chemical composition of cold pressed LPEO which fruit species cultivated in Metidja situated in Northen Algeria. The physicochemical properties are significant to assess the quality of the studied oil in order to valorise it. The obtained results were compared with those found in literature from other places to highlight the quality of the local LPEO.

II. Materials and methods

II.1. Plant material and essential oil extraction

The plant material (i.e. Lemon) was cultivated in the Mitidja which located in the North of Algeria. The lemon barks were gathered from Algerian fruit juice industry. LPEO was extracted by cold-pressing fresh lemon peels by mechanical press on an industrial scale. This technique is simple and recommended for this material [14]. The obtained LPEO was stored in closed amber colored bottle at room temperature.

II.2. Physicochemical properties of LPEO

The different organoleptic characteristics (appearance, colour and odour) of LPEO were determined by sensory evaluation.

The pH was determined using pH paper and an approximate value was obtained.

Specific gravity is defined as the ratio of the weight of the definite volume of oil to the weight of the equal volume of water at a particular temperature. Specific gravity is an important criterion of the quality and purity of oils. It was calculated by weighing a graduated measuring cylinder empty using analytical balance, filled with a volume of distilled water and filled with the same volume of LPEO, respectively. The EO specific gravity value was computed as given in equation (1):

$$Specific\ gravity = \frac{m2-m1}{m3-m1} \quad \textbf{(1)}$$

Where, m1 is weight of cylinder with essential oil (g); m2 is weight of empty cylinder (g); m3 is weight of cylinder with distilled water (g).

The refractive index is frequently used in determination of the identity, the quality and the purity of EOs [15]. The refractive index value of LPEO was determined at 20ºC by using the digital refractometer Hanna HI 96801.

The solubility of the LPEO was determined by mixing one volume of the EO in specified volumes of ethanol 96% in test tube.

The acid value expresses the number of milligrams of potassium hydroxide (KOH) required for the neutralization of the free acids present in 1 g of EO. The acidic value of LPEO was performed according to international standard [16].

LPEO determination of functional groups was investigated by direct attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). The analysis was performed using BURKER ALPHA FTIR spectrophotometer. A drop of sample was deposited directly on an ATR sample accessory provided with a diamond crystal. An infrared spectrum in transmittance mode was obtained. The background spectrum was performed by measuring the clean ATR crystal before each sample measurement. Spectral data obtained were plotted on a graph of transmittance (%) versus wave number (cm-1).

GC-MS is one of the golden standard techniques for analysing the volatile fractions of EOs [17]. The chemical composition of the LPEO was determined using Agilent GC 6890, equipped with Agilent MS D 5973 detector. The chromatographic separations were performed on a non-polar capillary column HP-5MS (5% Phenyl Methyl Siloxane) (30 m length × 0.25 mm inner diameter × 0.25 µm film thickness). High purity helium was used as the carrier gas at a constant flow rate of 0.5 ml/min. A 0.2 µl sample was injected in the split mode with a split ratio of 80:1 and the split flow rate of 0.5 ml/min. The oven temperature was maintained at 60 °C for 8 min after injection and then programmed from 60 to 250 °C at a rate of 2 °C/min, and subsequently held isothermal for 10 min. The detector was operating in the electron impact mode (70 eV). The temperatures of the injector, transfer line and ion source were set at 250 °C, 270 °C and 230 °C, respectively. Solvent delay time was 3.5 min. The obtained mass spectra were compared with data from Wiley 7 mass spectra library. The relative percentages of the constituent compounds were percentages from the GC peak areas based on the total ion chromatogram.

Retention indexes (RI) of each identified compound were calculated using the equation proposed by Van Den Dool and Kratz [18] in order to compare retention indexes of identified compounds with those

reported in literature. The compounds' retention indexes were calculated injection of a solution containing the homologous series of normal alkanes (C8 – C29) under the same conditions, as reported by van Den Dool and Kratz [18]:

$$RI = 100n + 100[\frac{tx-tn}{tn+1-tn}] \quad \textbf{(2)}$$

Where n is the number of carbon atoms in the n-alkane that elutes before the compound undergoes characterization, tx is the retention time of the considered compound, tn is the retention time of the alkane with n carbon atoms, and tn+1 is the retention time of the alkane with n+1 carbon atoms.

III. Results and discussion

III.1. Physicochemical properties of LPEO

The physicochemical parameters of LPEO are listed in Table 1.

Table 1. *Physicochemical properties of LPEO.*

Characteristics	Values
Appearance	Mobile, clear liquid
Colour	Pale yellow
Odour	Characteristics of fresh lemon pericarp
pH	6
Specific gravity	0.894
Refractive index at 20 ºC	1.475
Solubility in ethanol 96%	Miscible (1 LPEO:5 EtOH) ml
Acidic value (mg KOH/ g)	2.1

The extracted LPEO was noted as mobile and clear pale yellow liquid. It has lemon aromatic fragrance. These organoleptic characteristics are in the accordance with the international ISO 855 standard [19]. The colour and fragrance of the oil can be used in bath soaps, cosmetics and perfumery items. An approximate pH of LPEO is slightly acidic which supposed the richness of oil with acidic character compounds. The low acidity of oils is considered as neutralized and safe for making skin care products [20]. Specific gravity and refractive index serve as a means of assessing the purity and quality of the volatile oil as well as for identification [15,20]. LPEO exhibits specific gravity value of 0.894 which is lower than water. This result is within the range of specific gravity of volatile oils [15] and indicates that the extracted oil is highly pure [20]. LPEO specific gravity is higher than that Nigerian LPEO (i.e. 0.85) [21]. The measured LPEO refractive index of 1.475 is the same and very close with those obtained by Turkish and Nigerian lemon peel essential oils, respectively [2,21]. The refractive index value is in the accordance with the international standard [19]. Acid value was used to determine the acidity of oil. The lower acid value indicated the high quality of product [22]. The LPEO showed acidity level of 2.1 mg KOH/g which is higher than that Nigerian LPEO (i.e. 1.77) [21]. However, this value is still in accordance with international regulations to crude cold pressed oil which threshold is 4 mg KOH/g [23].

III.2. Spectral analysis: Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of LPEO is shown in Figure 1 and the functional groups associated with their respective wave numbers are listed in Table 2. The obtained results revealed the presence in LPEO of alkanes, alkenes, phenols and aromatics. LPEO FTIR spectrum exhibits similarities with the spectrum of Limonene. In fact, the presence of signals at 2917 cm-1, 1643 cm-1, 1375 cm-1, 886 cm-1 and 797 cm-1 in LPEO spectrum constitutes the important peaks of limonene [17,24] which is probably the major component in LPEO.

Table 2. *Results of LPEO FTIR analysis.*

Wavenumbers (cm^{-1})	Bonds	Functional groups
2917.64	C-H stretch	Alkanes
1643.45	C=C stretch	Alkenes
1436.26	C-H bend	Alkanes
1375.39	-OH bend	Phenols
885.87	C-H stretch	Aromatics
797.67	C=C bend	Alkenes

III.3. Gas chromatography-mass spectroscopy analysis

The LPEO was analysed by GC–MS to determine its volatile composition. Chromatogram and chemical analyses of the volatile constituents of the LPEO were illustrated and summarized in Figure 2 and Table 3, respectively. Thirty-nine different compounds representing 99.80% of the total composition LPEO are identified by GC–MS: 12 monoterpenes, 9 sesquiterpenes, 4 aldehydes, 8 alcohols, 3 esters, 1 ketone, 2 ether oxides, and 1 epoxide. The proportion of monoterpenes (92.79%) is higher than that of sesquiterpenes (2.38%). GC-MS analysis confirm the obtained FTIR spectrum that Limonene is the major component in the LPEO. Limonene (64.75%), β-Pinene (11.24%), γ-Terpinene (11.72%), α-Pinene (1.93%), and β-Myrcene (1.68%) were the major components in LPEO. Other compounds were also detected at low percentages. Limonene, β-Pinene and, γ-Terpinene

were also the major components with different percentages in cold pressed LPEO from Turkey [2,5], Brazil [8], Australia [13], Japan [25], Venezuela [14], and from Ivory Coast [12]. LPEO oxygenated compounds were found in amounts of 4.63%. Aldehydes were the most abundant oxygenated component. The main aldehydes were Geranial (1.68%) and Neral (0.96%). These results are slightly similar to Venezuelian [14] and Japanese [25] LPEO. Brazilan LPEO studied by Simas et al. [8] was composed with 2.2% of Geranial and 1.7% of Neral. Turkish LPEO contained only 0.15% of Geranial [5]. Neither Geranial nor Neral were found in the Turkish LPEO studied by Danila et al. [2]. It is difficult to compare the composition of LPEO because of the variation of chemical compounds and their percentage in the essential oil from natural sources may be influenced by many factors including climate, altitude, season of harvesting, geographical region of harvesting, chemotype, genotype, samples processing, plant part, extraction methods and duration of extraction process [2,4, 26-28]. According to Danila et al. [2], the presence of Limonene, Linalool, 4-Terpenol, α-Terpineol, Neryl acetate, Geranyl acetate and α-Pinene in the composition of LPEO provide an antimicrobial activity in the oil. Limonene, α -Pinene, β-Pinene and β-Myrcene have been also used extensively for the preparation of the perfume and flavouring agents, and medicines for local antiseptic and anesthetic use [29].

IV. Conclusion

The present work concerns to investigate quality and chemical composition of the LPEO extracted from lemon grown in the North of Algeria which lemon barks waste are collected from an Algerian fruit juice industry. LPEO physicochemical properties are in the accordance with international standard and regulation which indicates the good quality and the purity of the studied oil. Through GC-MS analysis, thirty-nine compounds were identified in the essential oil. Results shows a predominance of monoterpenes especially Limonene which prevalence was confirmed by FTIR spectrum. β-Pinene, γ-Terpinene, α-Pinene, and β-Myrcene are also majority compounds but in the less extent. The chemical composition reported in this work is globally similar with those in previous works. However, some variation of chemical compounds and their percentage in the studied LPEO are observed and could be influenced by several environmental factors. Presence of some components such as Limonene, Linalool, 4-Terpenol, α-Terpineol, Neryl acetate, Geranyl acetate, α-Pinene, β-Pinene and β-Myrcene lead to conclude that the studied LPEO have potentiality to be used in cosmetics and medicines which will be constituted the very interesting ways to manage lemon barks waste from Algerian fruit juice industries.

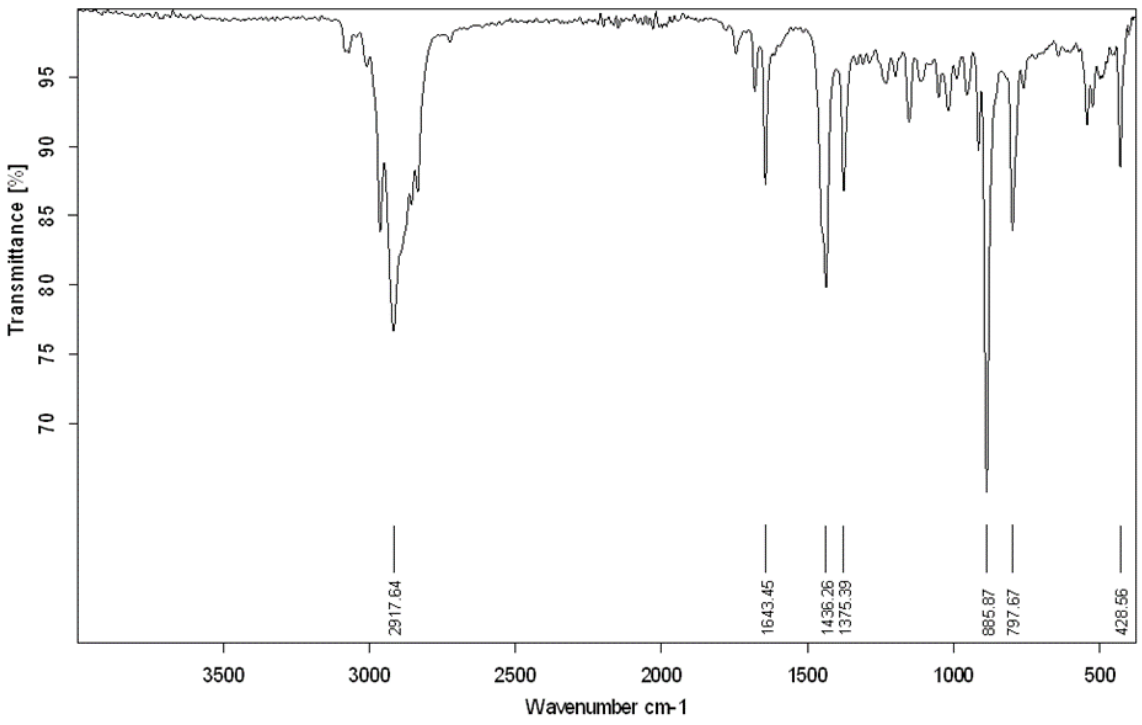

Figure 1. *FTIR spectrum of the LPEO.*

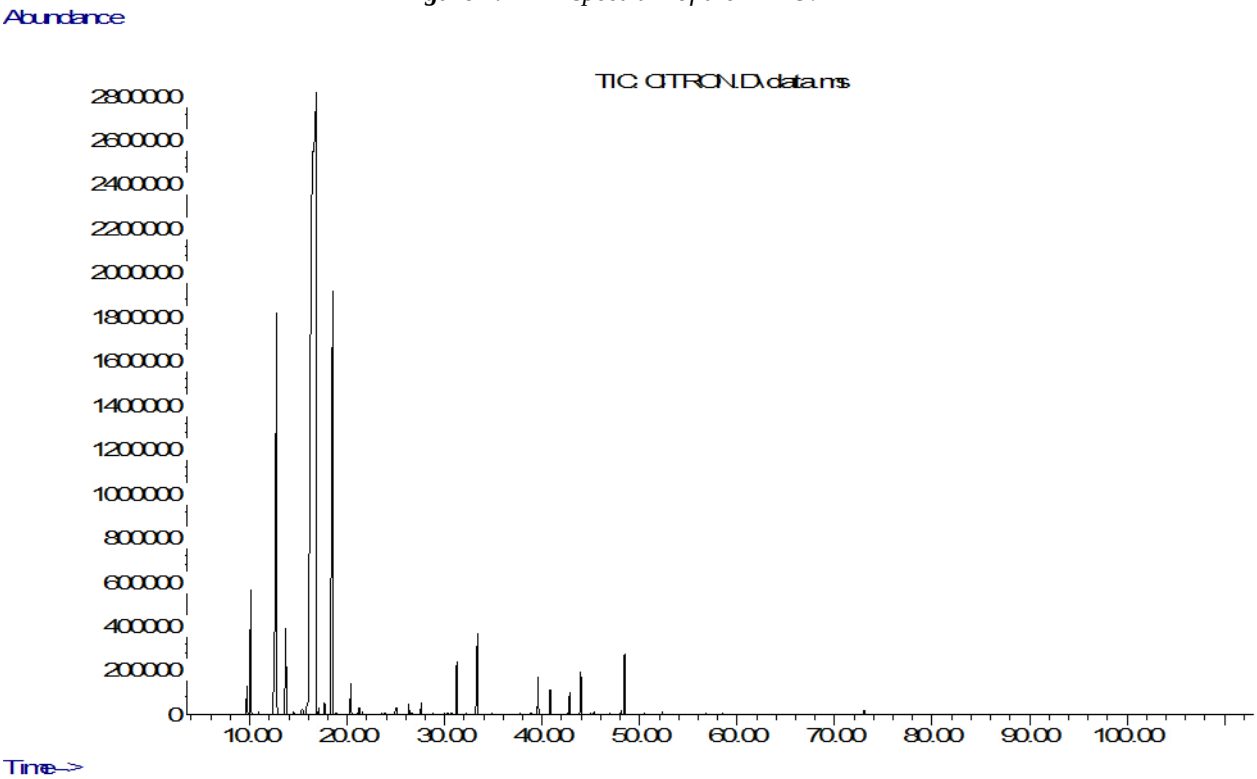

Figure 2. *Chromatogram of the LPEO.*

Table 3. *Chemical composition and relative abundance (%) of volatile compounds of LPEO obtained by GC–MS analysis.*

N°	RI	Components	Relative amounts (%)
1	936	α-Thujene	0.44
2	942	α-Pinene	1.93
3	953	Camphene	0.03
4	979	β-Pinene	11.24
5	993	β-Myrcene	1.68
6	1004	Delta-3-Carene	0.05
7	1016	α-Terpinene	0.20
8	1037	Limonene	64.75
9	1040	cis-Ocimene	0.08
10	1049	Trans-Ocimene	0.17
11	1061	γ-Terpinene	11.72
12	1066	Trans-4-Thuyanol	0.03
13	1087	α-Terpinolene	0.50
14	1099	Linalool	0.11
15	1123	Nonanal	0.04
16	1132	(1R,2S,4R)-1,2-Epoxy-p-menth-8-ene	0.02
17	1136	Limonene oxide	0.03
18	1153	(R)-(+)-Citronellal	0.18
19	1171	Menthol	0.16
20	1176	4-Terpinenol	0.03
21	1189	α-Terpineol	0.19
22	1228	Nerol	0.04

23	/	1,6,2,3-Dianhydro-4-deoxy-β- d-ribo-hexopyranose	0.03
24	1241	Neral	0.96
25	1255	Trans-Geraniol	0.03
26	1271	Géranial	1.68
27	1351	Citronellyl acetate	0.03
28	1362	Neryl acetate	0.60
29	1380	Geranyl acetate	0.39
30	1411	Trans-Caryophyllene	0.43
31	1431	Cis-α-Bergamotene	0.72
32	1448	α-Caryophyllène	0.02
33	1454	Trans- β-Farnesene	0.06
34	1482	b- Caryophyllène	0.02
35	1502	Trans-α-Bisabolene	0.06
36	1508	β-Bisabolene	1.03
37	1574	(Z,Z)-α-Farnesene	0.04
38	1680	α-Bisabolol	0.02
39	1823	Citroptene	0.09
Monoterpenes			92.79
Oxygenated monoterpenes			3.43
Sesquiterpenes			2.38
Others			1.20
Total			99.80

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