Essential Oil Extraction from Orange and Lemon Peel

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Abstract. The one-third of all food produced for human use is wasted each year which has made food waste a serious problem all around the world. Citrus juice manufacturing factories generate peel leftovers that can account for 50-60% of the weight of the fruit, contributing significantly to food waste. This study investigates the extraction of orange and lemon peel oils using the Soxhlet process and their analysis via gas chromatography. The aim is to identify and quantify key volatile compounds present in these oils, thereby putting the leftover peels to use. Fresh orange and lemon peels were transformed into zest and extracted using Hexane and Ethanol as solvent in a Soxhlet apparatus. Gas chromatography with a specialized column and detector unveiled major volatile compounds, including limonene, linalool, citral. These compounds contribute to the characteristic aroma and potential bioactivity of the oils. Orange peels displayed notably higher limonene content compared to lemon peels. This approach illuminated the chemical composition of the oils, offering opportunities in food, cosmetics, and fragrance industries. Orange peels contained 70-90% limonene, significantly more than lemon peels. The study concluded that peels harbor 28 volatile substances, with limonene responsible for their distinctive citrus scent. In sum, this research underscores the effectiveness of Soxhlet extraction, providing insights into orange and lemon peel oils' composition using Gas chromatography and applications.

Keywords. Extraction, Aroma compounds, Lemon Peel, Orange Peel, Extraction, Volatile Compounds.

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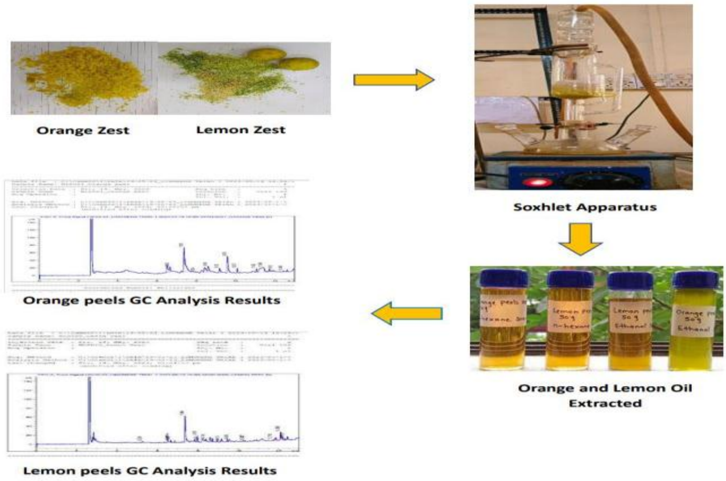
Graphical Abstract



1. Introduction

The fact that one-third of all food produced for human use is wasted each year has made food waste a serious problem all around the world. Citrus juice manufacturing factories generate peel leftovers that can account for 50-60% of the weight of the fruit, contributing significantly to food waste [1]. Fortunella, Eremocitrus, Clymendia, Poncirus, Microcitrus, and Citrus are six citrus fruit genera that are native to Asia's tropical and subtropical regions. Citrus, on the other hand, is the genus that contains the majority of commercially important fruits. Oranges, mandarins, limes, lemons, and grapefruits are just a few of the important fruits of the Citrus genus [2, 3]. The peel of the citrus fruit contains a high amount of the essential oil [4]. Essential Oils (EOs) are composite combinations of low-molecular-weight volatile compounds extracted from plant parts such as flowers, leaves, seeds, fruits, and stems of aromatic plants [5]. It is derived from plant material confined within a specific region of the plant or a specific component of the plant cells. The majority of the volatile chemical combination that makes up citrus essential oil is monoterpene hydrocarbon [4, 6]. The majority of the citrus essential oil is found in the flavado part of the peel, with smaller amounts found in the leaves, flowers, fruits, and seeds. Terpene hydrocarbons, oxygenated compounds, and non-volatile compounds are three fractions that can be roughly split into the more than a hundred different chemicals that comprise essential oils. The terpene fraction can make up 50 to 95% of the oil. Germicidal, antioxidant, and antibacterial properties, and anticarcinogenic activities are all present in the essential oil [7]. Some of the processes used to obtain essential oil include hydro-distillation, solvent extraction, supercritical fluid extraction, cold pressing, and microwave extraction. Recently, several advanced extraction techniques, including ultrasound, microwave, enzymatic and supercritical fluid extraction, were proposed by researchers to evaluate the relatively high volume of peels generated [8]. Some of the advantages of these advanced methods over conventional methods were less time duration, low energy requirement, less solvent requirement, and low carbon dioxide generation. The major constituent of citrus essential oil is limonene, a highly lipophilic cyclic monoterpene that accounts for 68-98% of the oil's weight and up to 4% of the weight of citrus peel waste. Limonene is a commonly used food preservation agent and is designated as a generally recognised as safe ("GRAS") additive in the Code of Federal Regulations due to its antioxidant properties and fragrance [1]. In actuality, limonene is essential to the global flavor and fragrance industry. Numerous sectors have taken an interest in the existence of limonene, a substantial component of the essential oils present in orange peel that has antibacterial, antioxidant, biological, and herbal fragrance. $C_{10}H_{16}$ is the empirical formula for the monoterpene limonene. It is a liquid when it is room temperature. Along with the racemic mixture dipentene, it also exists as the optical isomers D- and L-limonene. As a precursor to carvone or a-terpineol, limonene serves as an important industrial chemical [9].

The difficulties in disposing of trash from the juice business and fruits such as orange peels generally caused environmental contamination. To address this issue, citrus fruit peels can be utilized as raw material to extract essential oils for a number of residential and industrial applications [10]. Because orange peel contains biomaterials such as essential oil, pectin, and sugar, it may cause environmental problems if discarded, most notably water contamination. If potentially marketable active substances, such as essential oil, could be extracted, this concern may be transformed into a selling point [11]. Following extraction, the peel might be used as a dry, high-protein stock feed, increasing the potential profit for the orange juice industry. Essential oils are collected from fruit peels, flowers, leaves, stems, roots, and seeds. They are extremely concentrated compounds. These oils are frequently utilised in a variety of goods, including foods, pharmaceuticals, medicines, the fragrance industry, and cosmetics, for their flavour and their medicinal or odiferous effects [12]. Essential oils are known to be subject to conversion and degradation reactions since they are made up of a variety of various lipophilic and highly volatile components that come from a wide range of distinct chemical families. A review of the literature on essential oil stability found that oxidative changes and degradation reactions, which can result in changes that are relevant to both sensory and pharmacological properties, have seldom ever been thoroughly addressed [13]. Pires et al. reported that D-limonene can be obtained by extracting and separating essential oils contained in citrus fruits. Essential oils are particularly abundant in the peels of fruits, so it is efficient to extract essential oils from citrus peels. 70-90% limonene pertains to, in fact, essential oil component extraction and d-limonene. Studies on the separation of stimuli and evaluation and research on their physiological function activity are being actively conducted, −D-limonene included in citrus fruits is obtainable to extract essential oil as well as separate the oil ingredient [14].

Four provenances of Citrus aurantium L. var amara (sour orange) were used to produce peel, leaf, and flower oils, all of which were harvested from the identical pedoclimatic and horticultural circumstances. The GC-FID and GC-MS methods were used to analyse their chemical composition. The constituent percentages of the various provenances showed striking variances. According to the research proposed by Park et al., linalool/linalyl acetate was found in petitgrain, limonene was found in peel oils, and linalool/linalyl was linked to -pinene in neroli oils, all of which were used to describe this chemical variability [4]. Pires et al. reported that limonene was extracted from the tangerine peel using soxhlet extraction, a kind of simultaneous steam distillation and solvent extraction (SDE). In order to quantify the extracted d-limonene, a reversed-phase HPLC column was used in the HPLC study. The best extraction period in any solvent, according to the HPLC analysis results, was 2 hours, and the extracted levels of d-limonene from tangerine peel were 7.77 mg, 0.49 mg, and 0.28 mg in ethyl alcohol, n-hexane, and ether, respectively, per g of tangerine peel. Because ethyl alcohol produced the best yield when used as a solvent, polarity has a greater impact on extraction yield than boiling point [14].

After reviewing earlier studies on the essential oils of the orange and lemon peels, it is evident that limonene has anti-inflammatory characteristics. In fact, limonene inhibits the activity of polysaccharides that drive excessive production of pro-inflammatory cytokines (TNF-alpha) and nitrate oxide regulator of inflammation. Therefore, limonene appears to be a potent anti-inflammatory, particularly in cases of skin irritation [15, 16]. Overall, monoterpenes have a number of medicinal qualities, particularly insecticidal and anti-diabetic ones. It was shown that the monoterpenes in orange essential oil exhibit larvicidal activity against Aedes aegypti mosquito larvae in the fourth larval stage Culex pipiens [17, 18], which is the source of yellow fever [19]. Monoterpenes can imitate the qualities of insulin, promote its secretion by repairing dead pancreatic beta cells, or even further boost its secretion by living pancreatic beta cells in the case of type 1 diabetes, also known as insulin dependent diabetes [20, 11]. The essential oil of Citrus sinensis epicarp has lately received attention for its antioxidant properties, which were linked to the presence of phenolic compounds [21].

Fruits peel from industrial or domestic waste caused contamination of the environment. Extraction of essential oil from peel has several useful applications in industry. The purpose of this study is to investigate the limonene content of various citrus fruits, specifically orange and lemon. Limonene finds various applications as it is a popular additive in foods, cosmetics and cleaning products. The oil was extracted from lemon and orange peels using the Soxhlet technique and distilled to produce a concentrated combination of essential oil. The extracted combination was then sent for GC - FID characterization. The limonene concentration of orange and lemon peels was then established.

2. Materials and Methods

2.1 Materials

From different citrus fruit waste, lemon and orange peel were selected for this work. Hexane and ethanol are commonly used solvents for essential oil extraction due to their specific properties and advantages. Many essential oil constituents, like terpenes, are non-polar, making hexane an efficient solvent for their extraction. Hexane tends to extract a relatively narrow range of compounds, which can be advantageous when you want to target specific components in the essential oil. It has a low boiling point, making it easy to remove from the extract after the extraction process, leaving behind the essential oil. On the other hand, Ethanol has good solvating power for a broad range of compounds, including both polar and non-polar constituents present in essential oils. This allows for a more comprehensive extraction of various components. Compared to some other solvents, ethanol is less toxic and poses fewer risks when handled properly.

The chemicals used for this work were dilute nitric acid, n-hexane of laboratory grade, ethanol of laboratory grade, along with distilled water, Whatman filter paper. All chemicals were from Loba Chemicals. The glassware used for this work were round bottom flask, basket heater, Soxhlet extractor (along with siphon arm), condenser, measuring cylinder, beaker, funnel, specific gravity bottle, digital weighing scale.

2.2 Preparation of the Sample

Orange peels were cut into intermediate sized pieces (approximately 3*3 cm) and heated in an oven at a temperature of 100^0C for 20 min, 30 min and 40 min respectively. The oven was not preheated. Orange and lemon peels were grated to obtain the zest of fresh skin. Zest of fresh skin was ultimately used for the experimentation.

Fig. 1. Orange and Lemon Peels Zest

2.3 Extraction

The setup for Soxhlet extraction was arranged as shown in figure 2. Soxhlet extraction was a continuous, automated extraction method used to extract compounds from a solid sample with the helpe of a solvent. The apparatus used in Soxhlet extraction consisted of three main parts: the extraction chamber, a round-bottom flask containing the solvent, and a condenser. The solid sample, 50g orange zest, had been extracted and was placed in a porous thimble made of materials such as cellulose or glass microfiber, which fits into the extraction chamber. The appropriate solvent was chosen based on the nature of the compound that has been extracted. For example, if non-polar compounds like essential oils were the target, hexane might be used as the solvent. The round-bottom flask containing the solvent was heated, causing it to evaporate and rose as vapor. The vapor was then condensed in the condenser and dropped back into the solid sample in the extraction chamber. As the solvent dropped back into the extraction chamber, it gradually dissolved the target compounds from the solid sample. The dissolved compounds in the solvent gradually accumulated in the round-bottom flask as the process continued. This accumulation leads to an increase in the concentration of the target compounds in the solvent. The extraction process continued until the concentration of the target compounds in the solvent reached a saturation point. At this point, the process was considered completed. The solvent enriched with the extracted compounds was collected in the round-bottom flask for further processing and analysis.

2.4 Distillation

The solvent and oil separated when the mixture was heated in distillation at a steady temperature. The boiling points of the solvent was lowered than those of the majority of the active components in the essential oil. For n-hexane the boiling point was 68.7^0 C, and for ethanol it was 78.37^0 C. The simple distillation process continued in this manner until over 200 ml of the solvent was recovered in the beaker. The majority of the oil and a little amount of the unseparated solvent was present in the mixture that was left behind in the rounded bottom flask. Whatman filter paper was then used to filter the mixture to eliminate insoluble solid particles and obtain pure oil.

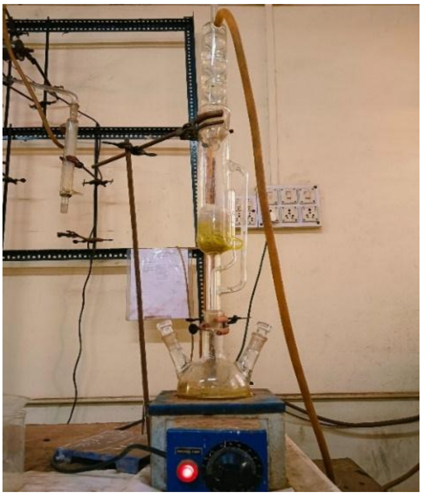


Fig. 2. Soxhlet Extraction

2.5 Characterization

The characterization of the distilled mixture was done by GC - FID process. The GC -FID analysis process was conducted in Wellia Labs, Pune, Maharashtra, India where the samples were provided and the lab after running the tests provided the results which are mentioned in the paper further.
By injecting 1.0 L of an essential oil and hexane solution into a chromatograph (HP Agilent 7890A), the chemical analysis of the produced essential oil was carried out. At a flow rate of 30 mL/min, nitrogen served as the carrier gas. The column is an HP 5 capillary column with a length of 30 m and an internal diameter of 0.32 mm. The stationary phase had a 0.25 m thickness. The oven's temperature was programmed to be 120 °C for 6 minutes before increasing by 10 °C/min to 260 °C in 5 minutes. The flame ionisation detector (FID) was employed in this work. Everything there was managed by a computer using the National Institute of Standards and Technology (NIST) database which allowed the identification of compounds.

3. Result and Discussion

The extracted essential oils were clear, orange yellow in colour for oranges and pale yellow green for lemons. Orange peel essential oil had a sweet, fruity aroma, while lemon essential oil had a fresh, beautiful lemon aroma as well as distinct pinene and terpinene aromas. Seven runs were conducted using various combinations of feed and solvent. The combination giving the best results was selected and a final run was performed.

3.1 Orange Peel Essential Oil

Table 1 presents a comparison of all the runs performed with orange peels zest.

Table 1. Comparison Table of Orange Peels Runs

	RUN 1	RUN 2	RUN 3	RUN 4	RUN 7
SOXHLET EXTRACTION					
Feed	30 g Orange Zest	50 g Orange Zest	50 g Dried Orange	50 g Orange Zest	50 g Orange Zest
Solvent (mL)	300 Hexane	300 Hexane	300 Hexane	300 Hexane	300 Ethanol
Time (min)	60	120	120	60	60
Temperature (degree C)	70	80	80	70	75
Density of Extract (g/mL)	0.84	0.85	0.99	0.90	0.72
DISTILLATION					
Time (min)	60	60	60	70	60
Temperature (degree C)	70	80	80	70	70
Separated Hexane (mL)	200	200	221	200	200
Density of Hexane (g/mL)	0.85	0.85	0.98	0.85	0.79
Density of Oil (g/mL)	0.89	0.97	0.62	0.96	0.89
Amount of Oil Extracted (g)	30	32	28	36.27	33
Yield of Extraction (%)	100	64	56	72.54	66

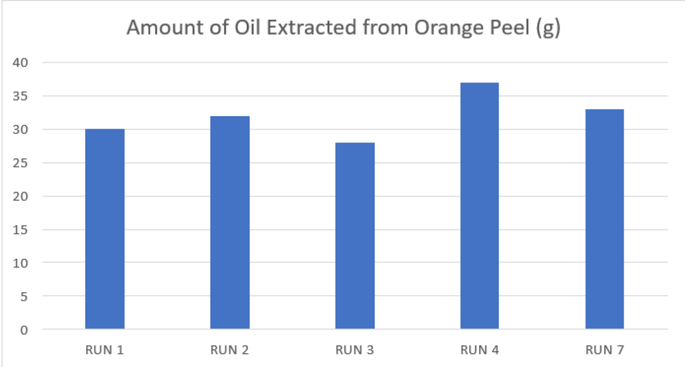


Fig. 3 Amount of Oil Extracted from Orange Peel (g) in Different Runs

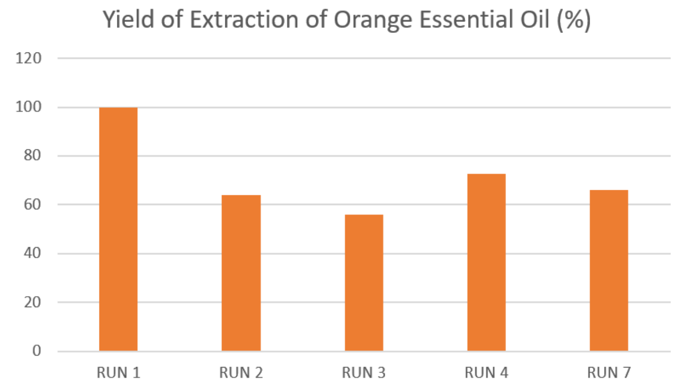


Fig. 4 Yield of Extraction of Orange Essential Oil (%) in Different Runs

The comparison between dried orange peel samples and orange peel zest samples shows how much less oil was extracted when dried orange peel samples were used in run 3. In run 3 oil obtained was 28 g. In run 1, 2, and 4 N-hexane was used as solvent and obtained 30, 32, and 36.27 g oil respectively as shown in figure 3. While in run 6, oil obtained was 33 g with ethanol as the solvent. Run 4 yielded the highest amount of oil, 36.27 g. Figure 4 shows a graph with the extraction yield shown on it. Since 100% yield is almost impossible after extraction and distillation, Run 1's 100% yield suggests that the oil's n-hexane level was exceptionally high.

In terms of output, runs 4 produces the best results for Orange Peels. In order to confirm the accuracy of all runs, multiple runs were taken with the same feed, solvent, time and temperature conditions. The following table 2 includes the results:

Table 2. Extraction Yield for Orange Peel (Run 4)

	RUN 4
Feed + Solvent	50g Orange Zest + 300 mL Hexane
Density of Oil	0.96 g/mL
Amount of Oil Extracted	36.27 g
Yield of Extraction (%)	72.54%

A percentage of the relative weight of the essential oil extracted from the peel weight was used to measure the extraction yield. The yield of the extraction, which is extremely high in proportion to the original weight collected, was 72.54% for orange zest as shown in Table 2.

For the GC-FID analysis, orange peel zest and n-hexane (RUN 4) was provided. The chromatogram displayed in Fig. 5 has been generated by the GC-FID analysis.

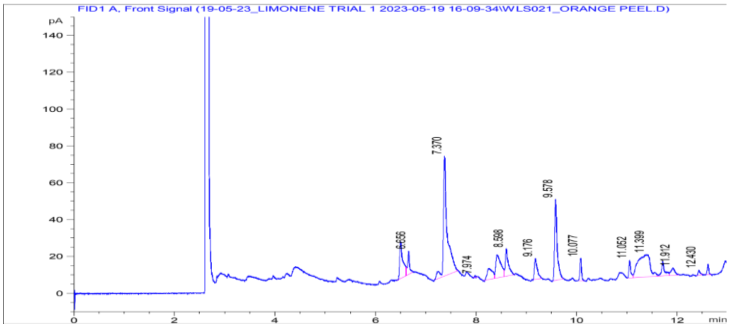


Fig. 5 GC - FID of Orange Peels

Table 3. GC-FID Analysis and Output for Orange Essential Oil

Compounds	Peels	Content (%)	Retention Time (minutes)
Limonene (Both Dextrorotatory and Levorotatory together)	Orange	80	7.370

Analyses have shown 28 volatile compounds in orange peel essential oil. Of them, 32% are terpene compounds, 50% are alcohol, and 18% are formaldehyde. GC – FID chromatogram of the orange peel oil extract displayed 16 identified peaks. The results, including the orders of elution as well as the relative peak areas, are in accordance with the NF ISO 855 standard where most of the components guarantee the quality of this essential oil, its origin and method of production. Orange peels had a much higher percentage of Limonene. Although it is expected that oranges will contain more than 90% limonene, due to water content in the solvent and flaws in the equipment, this percentage for orange peels has almost been lowered by 10%.

3.2 Lemon Peel Essential Oil

For Lemon Peels, Run 5 yields the best results in terms of output. Multiple runs were taken keeping the feed, solvent, time and temperature same, and yet same output as that of Run 5 was observed every time. The outcomes are shown in table 4 below:

Table 4. Comparison Table of Lemon Peels Runs

	RUN 5	RUN 6
SOXHLET EXTRACTION		
Feed	50 g Lemon Zest	50 g Lemon Zest
Solvent (mL)	300 Hexane	300 Ethanol
Time (min)	60	60
Temperature (degree C)	75	75
Density of Extract (g/mL)	0.80	0.78

DISTILLATION		
Time (min)	60	60
Temperature (degree C)	70	70
Separated Hexane (mL)	200	200
Density of Hexane (g/mL)	0.88	0.79
Density of Oil (g/mL)	0.95	0.84
Amount of Oil Extracted (g)	38	34
Yield of Extraction (%)	76	68

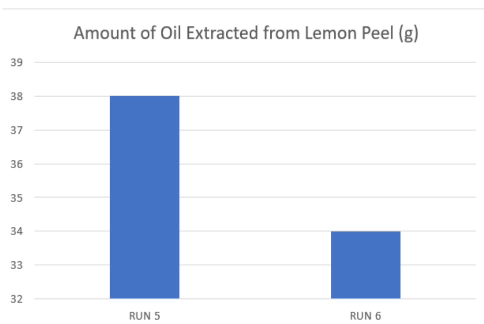


Fig. 6 Graph Representing the Amount of Oil Extracted from Lemon Peel (g)

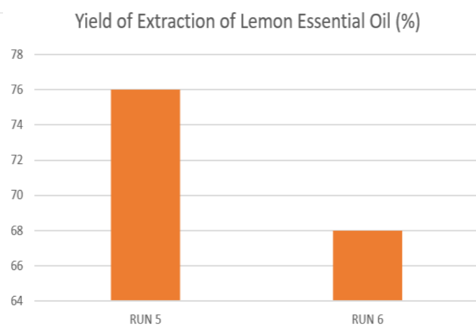


Fig. 7 Yield of Extraction of Lemon Essential Oil (%) in Different Runs

Table 5 shows that run 5 produced the most oil, 38 g and 76% yield, compared to any run. Whereas, run 6 produced only 34 g of oil and yield was 68%. The extraction yield was calculated as a fraction of the relative weight of the essential oil extracted from the peel weight. For lemon zest, the extraction yield, which is incredibly high in comparison to the initial weight gathered, was 76%. The main components of lemon peel essential oil, in addition to limonene, were β-pinene and γ-terpinene, which were present in amounts of 5-12% and 5- 10%, respectively. The lemon essential oil also contains the volatile substances sabinene, linalool, terpinen-4-ol, α-terpineol, octanal, and myrcene.

For the GC-FID analysis, lemon peel zest and n-hexane (RUN 5) was provided. The chromatogram displayed in Fig. 8 has been generated by the GC-FID analysis.

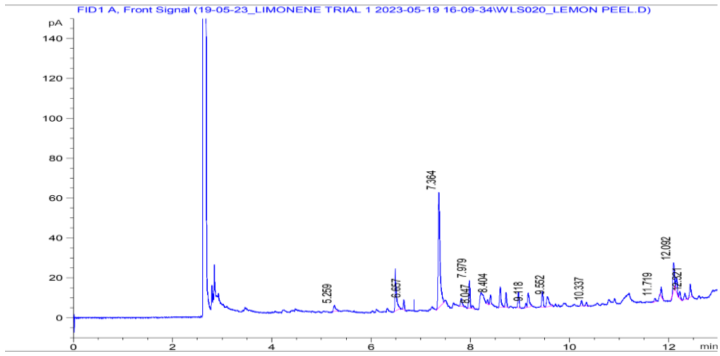


Fig. 8 GC - FID of Lemon Peels

Table 5. GC-FID Analysis and Output for Lemon Essential Oil

Compounds	Peels	Content (%)	Retention Time (minutes)
Limonene (D and L)	Lemon	70	7.364

As a reference method for separating volatile chemicals, lemon essential oil derived using the Soxhlet extraction process was analysed by GC. This is because the chemical composition of essential oils varies based on the season, the place of origin, and the extraction method. For a semi-quantitative method, the main chemicals were determined using a GC-FID analysis. The lemon peel oil extract's GC-FID chromatogram showed 13 recognised peaks.

3.3 Comparison of Orange and Lemon Peel Essential Oil

A comparative study between orange and lemon essential oils shows that orange peels yield a better amount of extract and density of oil in comparison to the lemon peels. Orange peels and lemon peels contain 80% and 70% of limonene, respectively. This is negligible in comparison to the range of 90%+ and 70–90% limonene that a good essential oil extraction should include [10]. This low proportion may be related to the extraction process, including the presence of water and other contaminants in the solvent. Additionally, the locally produced equipment's shortcomings are partly responsible for the lower amount of limonene found in each of these essential oils.

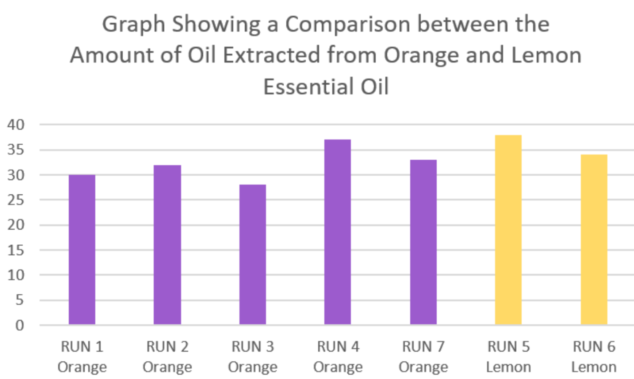


Fig. 9 Graph Showing a Comparison between the Amount of Oil Extracted from Orange and lemon Essential Oil

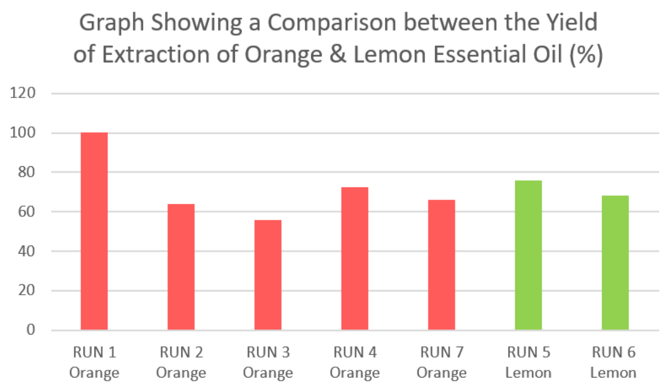


Fig. 10 Graph Showing a Comparison between the Yield of Extraction of Orange and Lemon Essential Oil (%)

Fig. 11 Run 4 and Run 5 Sample Bottles for Analysis

Santerre et al. studied detection of essential oils in citrus fruits peel and obtained 67% of the limonene content in lemon peel extract [22]. While in this study we obtained 70% of limonene from lemon peel with hexane solvent which was higher than reported values. Golmohammadi, et al. studied limonene extraction from citrus fruits peel by steam explosion method and obtained 77% of limonene from orange peel [23]. In this study we obtained 80% of limonene from orange peel using hexane solvent which was higher than reported value. In literature it was reported that the other major components of orange peel oil are linalool, β-myrcene, and α-pinene and that of lemon peel oil are limonene, β-pinene, and γ-terpinene [24]. Similar results we obtained in this work. Orange peels contain 28 volatile substances, of which limonene is believed to be responsible for giving it its unique smell that distinguishes it apart from other citrus fruits. Out of 28 volatile compounds present, 32% are terpene compounds, 50% are alcohol, and 18% are formaldehyde. The main components of lemon peel essential oil, in addition to limonene, were found to be β-pinene and γ-terpinene, which were present in amounts of 5-12% and 5- 10%, respectively. The lemon essential oil also contains the volatile substances sabinene, linalool, terpinen-4-ol, α-terpineol, octanal, and myrcene.

4. Conclusion

The Soxhlet extraction method is effective in obtaining orange and lemon peel oils. From all the runs conducted the 4th and 5th runs yielded the highest amounts of oil 36.27g and 38g respectively. Dried orange peel samples yield less oil than orange peel zest samples as limonene is a volatile component which gets vaporized on heating the peels. N-hexane is a better solvent for extracting oil from orange and lemon peels than ethanol which is evident by the multiple runs conducted. Limonene is the major component of both oils responsible for the characteristic aroma, but it is present at a higher concentration in orange peel oil. Through GC-FID characterization, it was evident that limonene content in orange peel oil was 80% and that in lemon peel oil it was 70%. The combination of the Soxhlet extraction method and GC-FID analysis has played a major role in unraveling the complicated chemical composition of the essential oils derived from orange and lemon peels. The highest yield of 76.54% limonene was obtained from orange peel as compared to lemon peel with hexane solvent. The significance of extraction parameters becomes clearly evident, with factors like solvent selection and extraction duration over oil yield.

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