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# Extraction of Essential Oil from Sour Orange (*Citrus aurantium*) Peels and Evaluation of its Physico Chemical Characteristics

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**Abstract**

The present work was carried out to obtain essential oil from the sour orange peel (*Citrus aurantium*) which is rich in essential oil through steam distillation. Extraction using distilled water was the control treatment and other treatments were carried out by using 3%, 5%, and 7% $NaHCO_3$. This study determined the yield% of essential oil in four different treatments in freshly extracted essential oil. The Physico-chemical properties of essential oil were determined in freshly extracted essential oil .In performance, T3 (5% $NaHCO_3$) was the best yield% (w/w) with a value of 3.15%. According to this study, using $NaHCO_3$ for essential oil extraction from sour orange peel resulted in a higher essential oil yield than distilled water. The extracted essential oil in sour orange peel has distinct pale yellowish color with a fresh and tangy smell. T3 exhibited that distinct color and odor. Based on the yield% and Physico-chemical analysis, the essential oil extraction using 5% $NaHCO_3$ was the best treatments followed by treatment 4 (7% $NaHCO_3$), treatment 2 (3% $NaHCO_3$) and treatment 1.

***Key Words:*** *Sour orange peel, Extraction, $NaHCO_3$, Essential oil*

## Introduction

Fruits with nutraceutical and medicinal properties are gaining importance in the modern food industry. Among many fruit trees, Citrus is an extremely important bearing genus in this regard. It includes well-known species like orange, lime, mandarin, and lemon, as well as many other underutilized species. Oranges are among the most popular fruit in the world due to their pleasant taste (Nguyen *et al*., 2009), as well as their nutritional value. So, orange fruits have long been part of the human diet due to their nutritional and medicinal properties.

A large amount of wet solid waste is generated because of the widespread consumption of orange juice. Orange juice is one of the popular beverages today. About 50-60% of the processed fruits are converted into citrus peel, which is made up of peels, seeds, and membrane residues. Fruit waste is increasing dramatically as the production of processed fruit juice increases (Garg, 2017). Orange peels are discarded inside waste containers even in homes because people prefer the orange pulp for its juicy pulp and discard the peel because it does not taste as good as the pulp. Furthermore, citrus waste is disposed of in a variety of ways. Waste discharged into lakes may cause pollution and extinction of aquatic life, especially if the body of water is insufficient to dilute the waste properly (Sharma *et al*., 2017).

This waste is caused to pollute the environment if not properly handled. That waste may affect both economic and environmental problems such as high transportation costs, lack of dumping sites, and accumulation of high organic content material (Tripodo *et al*., 2008). As a result, it is critical to treat citrus waste in food industries and other areas in a systemic manner. By producing by-products from these wastes, they can be effectively disposed. This waste primarily consists of orange peels. The peels have numerous oil-producing glands that contain significant amounts of citrus oil (Lota *et al*., 2000). As a result, rather than discarding these peels as solid waste, they can be used to extract oil (Virot *et al*., 2008).

Citrus essential oils have gotten the most attention because of their broad-spectrum insecticidal, antibacterial, and antifungal properties, as well as their high yields, aromas, and flavors (Bora *et al*., 2020). Citrus fruits have a high nutritional value, as well as high levels of elemental bioactive compounds, such as phenols, flavonoids, limonoids, essential oils, and vitamins, particularly vitamin C and carotenoids, which have numerous health benefits. These components give this fruit its distinct flavor and aroma, resulting in a more balanced and tasty diet. Above all, citrus is beneficial in the treatment and prevention of many diseases.

Citrus fruits are high in essential oils as well as other nutrients like vitamin B9, vitamin C, potassium, flavonoids, coumarins, pectin, and dietary fibers (Bora *et al*., 2020). Citrus peel oil was chosen for extraction because it has a high potential for further commercialization. Essential oils are aromatic compound mixtures that are widely used in perfumes, food, pharmaceutical, and other industries. Essential oils are primarily composed of monoterpene hydrocarbons, sesquiterpenes, and their derivatives such as esters, alcohols, and aldehydes (Toan *et al*., 2020).

Citrus essential oil also has antibacterial, antifungal, and insecticidal properties (Burt, 2004). Essential oils from citrus peels are very complex matrices composed of many compounds of various chemical classes that are primarily separated into two parts: the volatile part and the non-volatile part. Essential oils are liquids that are clear and rarely colored, and they are soluble in organic solvents that have a lower density than water (Palazzolo *et al*., 2013).

All citrus trees belong to the genus Citrus, the family Rutaceae. Bitter ("sour") oranges refer to *Citrus aurantium* L. and are hybrid between *Citrus maxima* (Pomelo) and *Citrus reticulate* (Mandarin). This plant is native to southeastern Asia (Anwar *et al*., 2015). *Citrus aurantium* is a Latin name for bitter orange (Moonaz *et al*., 2010). Bitter orange is well-known for its use in the treatments of anxiety, lung, and prostate cancers, gastrointestinal diseases, and obesity. *Citrus aurantium* essential oils contain bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, and anti-anxiety properties (Kacaniova *et al*., 2020).

Bitter orange consists of large amount of active constituents. Those active constituents can be found in bitter orange juice, flowers, seeds, leaves as well as peels. Active constituents in bitter oranges provide many biological effects, such as antioxidant, antimicrobial, anti-cancer, anti-diabetic, etc (Maksoud *et al*., 2021). Other than therapeutic applications, *Citrus aurantium* is used in the food production industry also. The food applications of bitter oranges are *Citrus aurantium* juices, wines, jams, canned fruits, and dried fruits. Those various applications of *Citrus aurantium* fulfill the better utilization of bitter orange and thus extend its industrial chain. The development of those value-added bitter orange products can partially overcome the defects of seasonality and the short life of fresh bitter oranges, and meet the nutritional requirements of novel fruit products from consumers (Liu *et al*., 2022).

The essential oil was extracted from citrus peel by using different methods such as steam or hydro distillation, maceration, solvent extraction, soxhlet extraction, cold pressing, supercritical fluid extraction, microwave-assisted extraction, etc. Those extraction methods can be divided into two types. They are traditional extraction methods (conventional methods) and non-conventional extraction methods. Conventional methods, which use organic solvents, necessitate long extraction time, large amount of energy, and other resources, posing as much of an environmental risk (Chemat *et al*., 2019) as the biodegradable properties of citrus waste already do. Because of the drawbacks of traditional extraction methods, the wide availability of citrus waste, and the emerging need to reduce environmental impact, there has been an increase in interest in the responsible management of this type of waste (Da *et al*., 2018). Furthermore, non-traditional technologies known as "green extraction methods" has gained importance. These are the most sustainable methods for obtaining bioactive compounds from citrus residues, allowing for better resource utilization. Their main advantages are improved performance, lower energy, time, and other resource expenditure, and the use of few or no organic solvents (Anticona *et al*., 2020).

## Materials and Methods

### Preparation of Sour Orange peel sample

The Sour Oranges were washed with clean water and peeled thinly. Thin outer peels were cut into small square shape cubes. Then those peels were ground into formed a fine particles of peels without drying.

### Prepared Sour Orange peel sample transferred into the Extraction

Kjeldahl apparatus was used for the extraction of essential oil from sour orange peels fine particles.

### Extraction of Essential oil with different treatments

The essential oil was extracted by using four different treatments. After the sample was transferred into the 500 ml flat bottom flask, the flask was connected to the Kjeldahl apparatus. 50ml graduated cylinder was used as the receiver. Then the mixture was distilled rapidly by heating it to 180ºC for 2 hours. The steam was generated from the boiling water (as the flask was heated) the volatile oil was extracted which was condensed as part of the steam into the distillation flask as it was passed through the cooling system.

T1 – Extraction with Distilled water (control): 150g sour orange peel particles + 250 ml distilled water
T2 – Extraction with 3% $NaHCO_3$: 150g sour orange peel particles + 7.5g $NaHCO_3$
T3 – Extraction with 5% $NaHCO_3$: 150g sour orange peel particles + 12.5g $NaHCO_3$
T4 – Extraction with 7% $NaHCO_3$: 150g sour orange peel particles + 17.5g $NaHCO_3$

**Separation of Essential oil**

The distillate (a mixture of oil and water) was poured into a separating funnel where the mixture separated into two layers. Then 10ml of n-hexane was added into the separating funnel and the stopper was placed. Then the separating funnel was shaken while releasing the pressure that may form in the funnel constantly. After that funnel was let into a stand for a 5 minutes for clear separation. The stopcock of the funnel was opened and the aqueous phase was drained into a 100ml Erlenmeyer flask. The stopcock was closed when the organic phase reaches it and it was recovered into a separate container. The process was repeated for twice using 5ml of n-hexane. Then needed to evaporate the hexane which in the beaker to isolate the oil using water bath. It was begun to evaporate at about 62.5 ºC. Finally, the thermometer was placed in the solution to monitor if it is hexane left or not. If the thermometer reached more than 80 ºC so then decide to stop evaporation. The extracted pure essential oil was placed in an airtight bottles.

**Characterization of the Essential Oil**

After the oil was extracted, it was characterized to ensure that the extracted liquid was indeed oil. The essential oil extracted from Sour Orange peels under optimal processing conditions was characterized by determining the physico-chemical properties.

**Statistical Analysis**

Each formulation was replicated in experiments and they were in Complete Random Design (CRD). Analysis of Variance (ANOVA) (α=0.05) and mean separation was done with Turkey's studentized test. Physio-chemical analysis and storage studies were done through Minitab 17 software statistical package

**Results and Discussion**

**The Yield of Extracted Essential Oils from Sour Orange Peel Samples**

**Table 1** Yield % of freshly extracted essential oil samples

| Treatment | Yield %(W/W) |
|---|---|
| **T1** | $1.46 \pm 0.22^{c}$ |
| **T2** | $2.09 \pm 0.01^{b}$ |
| **T3** | $3.15 \pm 0.04^{a}$ |
| **T4** | $2.52 \pm 0.04^{b}$ |

The values are means of the triplicates ± standard error.
Means values with the same letters within the same column do not differ significantly at 5% level.

According to the results, essential oil yield % was high in the T3 (5 % $NaHCO_3$) treatment. The maximum essential yield was 3.15%. Mainly essential oil yield % was less in the first treatment which the extraction was done using distilled water. That yield was 1.46%. When the $NaHCO_3$ percentage was increased from 3% to 5%, the essential oil yield% also increased, but again at T4 (7% $NaHCO_3$), the oil yield % was decreased than T3.

**Color**

The color was determined by observing the samples visually. T1 (control) and T3 (5% $NaHCO_3$) essential oil samples were pale yellowish-white in color. T2 (3% $NaHCO_3$) and T4 (7% $NaHCO_3$) essential oil samples were pale reddish brown in color.

**Odor**

All treatments, T1, T2, T3, and T4 were given a fresh and tangy smell.

**Solubility**

In all treatments, T1, T2, T3, and T4 essential oil samples were insoluble in water.

**Chemical Characteristics of Freshly Extracted Essential oil**

The chemical characteristics of extracted essential oil such as saponification value, acid value, free fatty acid value, ester value, pH, and refractive index of the freshly extracted essential oil revealed there was significance difference between the treatments of essential oil at 5% level of significance.

**Table 2** Chemical characteristics of freshly extracted essential oil samples

| Treatment | Saponification value (mg KOH/g) | Acid value (mg KOH/g) | Free fatty acid content (mg KOH/g) | Ester value (mg KOH/g) | pH | Refractive index |
|---|---|---|---|---|---|---|
| **T1** | 24.52±0.20$^{d}$ | 4.10±0.06$^{a}$ | 3.06±0.05$^{a}$ | 20.42±0.21$^{d}$ | 6.76±0.01$^{c}$ | 1.35±0.006$^{a}$ |
| **T2** | 41.54±0.17$^{c}$ | 3.87±0.03$^{b}$ | 2.43±0.07$^{b}$ | 37.67±0.14$^{c}$ | 6.97±0.07$^{c}$ | 1.25±0.006$^{b}$ |
| **T3** | 42.67±0.11$^{b}$ | 3.47±0.03$^{c}$ | 2.09±0.02$^{c}$ | 39.19±0.14$^{b}$ | 7.27±0.08$^{b}$ | 1.21±0.006$^{c}$ |
| **T4** | 44.57±0.21$^{a}$ | 3.07±0.04$^{d}$ | 1.94±0.003$^{c}$ | 41.49±0.21$^{a}$ | 8.12±0.003$^{a}$ | 1.02±0.006$^{d}$ |

The values are means of the triplicates ± standard error
Means values with the same letters within the same column do not differ significantly at 5% level.

While increasing the $NaHCO_3$ concentration from 3% to 7% for essential oil extraction, the saponification value of freshly extracted essential oil from sour orange peels was increased from 41.54 mg KOH/g to 44.57 mg KOH/g. when the $NaHCO_3$ concentration was raised from 3% to 7%, the acid value of the extracted essential oil decreased from 3.87 mg KOH/g to 3.07 mg KOH/g. According to the results, treatment 1, which extraction of essential oil using distilled water, contains the least mean ester value (20.42 mg KOH/g). The mean ester value of treatments 2, 3, and 4 were found as 37.67, 39.19, and 41.49 mg KOH/g respectively
According to the results, pH value (Figure 4.6) was high in T4 (7% $NaHCO_3$) essential oil. The highest mean pH value is 8.12. pH value was less in T1 (control) than in others. The least pH value is 6.76. pH value was increased, when increasing $NaHCO_3$ concentration from 3% to 7%. According to the results, mean value of refractive index was high in treatment 1 (control). The least refractive index was in treatment 4 (7% $NaHCO_3$).

## Conclusion

The results of this particular study revealed that in the extraction of essential oil using steam distillation, the highest yield % of essential oil was obtained from T3 (5% $NaHCO_3$), 2 hours of distillation in sodium carbonate, therefore, the concentration of $NaHCO_3$ aids in the extraction of essential oil from the plant tissues of the sour orange peel. This research revealed that using $NaHCO_3$ for essential oil extraction, provided a higher yield than using distilled water. According to the results, the yield % was increased in the following manner. That is, $T1<T2<T4<T3$.Based on chemical characteristics, saponification value was increased according to $T1<T2<T3<T4$, 123.48mg KOH/g, 151.95mg KOH/g, 164.9mg KOH/g, 170.33 mg KOH/g respectively. Ester value was high in treatment 4 (7% $NaHCO_3$). Treatment 1 had the highest mean value for acid value and free fatty acid content than other treatments. Based on the Yield %, physical and chemical, the essential oil extraction using 5% $NaHCO_3$ was the best treatment compared to other treatments.

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