Characterization of Limonene Extracted from Citrus species fruit Peels via Distillation.

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ABSTRACT

The extraction and characterization of limonene from *Citrus species*: sweet orange (*Citrus sinensis*), lemon (*Citrus limon*) and lime (*Citrus aurantifolia*) fruit peels form the basis of this work. The *Citrus* fruits (sweet orange, lemon and lime) were obtained from Eke Ekwulobia market, in Aguata LGA of Anambra State which is in the Southeastern part of Nigeria. The peels were removed from the fruits and limonene was extracted from the peels of the three different fruits through simple distillation method. The results for the percentage yields of limonene from the different citrus peels showed that sweet orange peels have the percentage yield of 1.13%, lemon peels have the percentage yield of 0.76% and lime peels 0.69%. The limonene was characterized and the results showed that the limonene has citrus aroma, neutral to both blue and red litmus papers, changed the colour of potassium permanganate from purple to brown which is a positive test for unsaturation, reacted with conc. H_2SO_4 and $CHCl_3$ to form a red interface which is a positive result for terpenoids and finally it is insoluble in water. From the results obtained, we can conclude that limonene can be extracted from citrus peels using simple distillation method. Since limonene is an essential product, it is recommended that people should engage in its production to reduce waste and make wealth.

Key words: Limonene, *Citrus*, Peels, Distillation, Terpenoid.

INTRODUCTION

Citrus (*Citrus spp. L.*; family: *Rutaceae*) is one of the most widespread tree fruit crops with genera all over the world. Some important fruits of the genus citrus are sweet orange (*Citrus sinensis*),

lemon (*Citrus limon*), lime (*Citrus aurantifolia*), grapefruit (*Citrus paradisi*), citron (*Citrus medica*), shaddock (*Citrus grandis*), tangerine (*Citrus reticulate*) and sour orange (*Citrus aurantium*). Orange, mandarins and lemon trees are the three most important and abundant *Citrus species* in the world (Janati*et al.*, 2012), of which orange accounts for over 60% of the entire citrus crops (Aslin, 2014). They are mainly grown in tropical and sub-tropical areas of South-East Asia, in the Mediterranean countries of Europe and North Africa, in America, South Africa and Australia (Aslin, 2014).

Citrus sinensis which originated from southern China but currently cultivated worldwide is a small evergreen tree with height between 7.5m-15m (Ehler, 2011). The fruit, which may be globose or oval is usually 6.5 to 9.5 cm wide, and ripens to orange or yellow colour. Anatomically, the fruit consists of two distinct regions: the pericarp also called the peel, skin or rind, and the endocarp, known as pulp or juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate (Goudeau *et al.*, 2008). Citrus sinensis is a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium (Angew, 2007).

Citrus limon is an evergreen tree usually 2.5-3m in height with yellow edible fruits. The berry fruit is oval in shape with apical nipple, colour changes from green to yellow during ripening; the pulp forms 8-10 segments which contains acidic juice. The fruit consists of three parts; the exocarp (peel) which is a thick outer shell (with several oil glands) that turns yellow at maturity and in some varieties remains green. The middle part (mesocarp or albedo), a white spongy part rich in pectin and the inner part (a juicy endocarp) which contains organic acids, sugars and water (Mabberley, 2004). Its economic importance includes the nutritional and health benefits of its fruit juice, plus the medicinal and cosmetic benefits of its essential oil (Salem *et al.*, 2021).

Citrus aurantifolia is a small perennial evergreen tree grown for its sour fruit. The lime tree is irregularly branched and possesses sharp spines and produces small, cupped white flowers and yellowish-green fruits which are round or egg-like in shape. Lime trees can reach 5m (16ft) in height and can produce fruit for many years (CABI, 2014). The fruit has high vitamin C content and abundant macro and micronutrients (Liu, Heying & Tanumihardjo, 2012). Although the juice

is the main commercial product of limes, essential oil is extracted from it for the food industries; limonene, an oil derivative of lime, is also used in the cosmetics industry and in household cleaning products such as soaps and detergents (Chisholm, Wilson & Gaskey, 2003). Citrus fruits have been used as traditional medicinal herbs in several Asian countries, such as China, Japan and Korea. Derivatives of lime are widely used for their antibacterial, anticancer, antidiabetic, antifungal, anti-hy-pertensive, anti-inflammation, and antioxidant properties (Narang and Jiraungkoorskul, 2016).

Limonene is a hydrocarbon classified as a monoterpene and can be extracted from the peels of citrus fruits. Zema *et al.* (2018) revealed that limonene accounts for 32-98% of the essential oils found in citrus fruits, and is one of the chemicals responsible for their distinctive aroma. It is often referred to as (D)-limonene, which is its main chemical form but exists as (R)-limonene in orange and (S)-limonene in lemon. Several studies have shown that limonene has the potential for applications in many areas such as in the manufacturing of chemicals used in flavourings and fragrances, paints, cosmetics, cleaning agents and insect repellants (Njoku and Evbuomwam, 2014).Similarly, Farhat *et al.* (2011) revealed that essential oils such as limonene are predominantly used to add desired aroma to perfumes, cakes, ice creams, carbonated soft drinks, and room air fresheners. However, the usage of this essential oil is dependent on the cultivar, the plant matrices and method of extraction (Veillet*et al.*, 2010). The extraction of limonene from citrus peels have been reportedly performed using several approaches ranging from traditional Soxhlet method (Loprestoet *al.*, 2014), mechanical pressing, steam distillation, microwave steam diffusion mechanism (Farhat *et al.*, 2011), solvent extraction method (Cheong *et al.*, 2012), and several other methods as found in literatures. However, distillation is the most widely used methods for the extraction of limonene from citrus peels and this forms the basis for the present study which is aimed at extracting limonene from orange, lemon and lime peels.

MATERIALS AND METHODS

Sample Collection and Preparation

Three citrus fruits: sweet orange (*Citrus sinensis*), lemon (*Citrus limon***)** and lime (*Citrus aurantifolia*) used in this research were purchased from Eke Oko market in Orumba North L.G.A. of Anambra State. The citrus fruits were peeled using a grater to obtain small pieces of the peels.

Extraction of Limonene from the Peels

A simple distillation apparatus was set up in the lab for the extraction and 200g of each fruit peels were weighed. Extraction was carried out individually for each of the peels. After weighing the peels, they were put in the distillation flask and distilled water was added in such a way that it slightly covered the peels. The tap that supplied water to the condenser was then turned on before the Bunsen burner was lit on to heat the flask, and the distillate was collected with test tubes. After cooling the distillate, the oily product (limonene) separated from the water by forming two separate layers and was recovered with the aid of a syringe.

Percentage Yield

The percentage limonene yield of the peels of the three different fruits (sweet orange, lemon and lime) was obtained using the standard formula below.

Yield (%) = (mass of oil / mass of citrus peel) x 100

Test for Terpenoids

Limonene is a terpenoid and therefore test for terpenoids was carried out to confirm whether the product extracted from the peels is a terpenoid or not. This was done using the Harborne (1998) method in which 5mL of the extract was mixed with 2mL of chloroform and 3mL of concentrated sulphuric acid. A reddish colouration at the interface showed positive result for the presence of terpenoids.

Test for Unsaturation

This was done using the standard test for unsaturationknown as Baeyer's test. Limonene is an unsaturated hydrocarbon and this test was carried out to confirm whether the extracted product is unsaturated or not. The extract (5mL) was added into a test tube and few drops ofpotassium permanganate ($KMnO_4$) was added and shaken very well. The purple colour of the $KMnO_4$ disappeared and a brown precipitate was formed. This showed a positive result for unsaturation.

Test for acidity

The extract was tested using damped blue and red litmus papers. Two drops of the extract was put on both blue and red litmus papers and no colour change was observed. This means that the extract is neutral and not acidic nor alkaline.

Solubility test

The extract (2mL) was added to 100mL distilled water in a test tube and shaken vigorously. Two distinct layers appeared after a second showing that the extract was insoluble in water.

Physical tests

Physical tests were also carried out to check the colour (this was done by observing the colour with the eye) and aroma (smelling with the nose) of the extracts.

RESULTS

Results for the percentage yields of the extracts of the three citrus peels are given in table 1 below.

Table 1: The Percentage Yield of Limonene by the Three *Citrus species*

Samples	**Percentage Yield (%)**
Orange peels	1.13
Lemon peels	0.76
Lime peels	0.69

Results for the characterization of the extracts are shown in table 2 below.

Table 2: Characterization of Limonene in the three *Citrus species*

Tests	Citrus sinensis	Citrus limon	Citrus aurantifolia
Colour	Colourless	Colourless	Colourless
Odour	Orange aroma	Lemon aroma	Lime aroma
Acidity (Two drops of extract on damp red and blue litmus papers)	Neutral	Neutral	Neutral
Unsaturation (product + $KMnO_4$)	Colour changes from purple to brown (positive)	Colour changes from purple to brown (positive)	Colour changes from purple to brown (positive)
Terpenoids (Product + Conc. H_2SO_4 + $CHCl_3$)	Red interface formed (positive)	Red interface formed (positive)	Red interface formed (positive)
Solubility in H_2O	Insoluble	Insoluble	Insoluble

DISCUSSION

The results for the percentage yield of limonene from citrus peels showed that orange (*Citrus sinensis*) peels have the highest limonene yield of 1.13%. In 2022, Swathanthra and Naik reported a limonene yield of 0.96% from *Citrus sinensis* while Wani *et al.*, in 2021 reported a yield of 1.44%. The lemon (*Citrus limon*) peels used in this study gave a 0.76% yield of limonene, but Ayush *et al.*, in their research project in 2016 reported a percentage yield of 1.93% while Wani and his team in their work in 2021 reported a 1.80% yield of limonene from *Citrus limon*. Lime (Citrus aurantifolia) peels used in this study gave the least limonene yield (0.69%) among the three Citrus species used. This limonene yield is small compared to the limonene yield reported by Tran *et al.*, in 2021 which was 1.15%. These differences in percentage yield of limonene from the three citrus species may be due to difference in soil, weather, location and time of harvest.

The results for the characterization of limonene showed that limonene has a citrus aroma depending on the source of the limonene. Limonene produced from orange has an orange aroma this is in agreement with the work of El-Ishaq *et al*, in 2011. That from lemon has a lemon aroma and this is in agreement with the report of Uwidia and his friends in 2020. Limonene from lime has a lime aroma as well. The extracted limonene is colourless and did not change the colours of damped blue and red litmus papers which mean that it is a neutral compound. The test for unsaturated conducted with $KMnO_4$ (Baeyer's test) showed a positive result where limonene reduced the purple colour of $KMnO_4$ to brown with the formation of a manganous precipitate. The test for terpenoids as proposed by Harborne (1998) was also positive where the limonene reacted with conc. H_2SO_4 and $CHCl_3$ to form a red colouration at the interface, which shows that the limonene extracted is a terpenoid. The extracted limonene is also insoluble in water.

RECOMMENDATIONS

Extraction of limonene from citrus peels can be a good way of creating wealth from waste and it is recommended that more attention be given to it.

A more efficient way of extracting limonene from citrus peels should also be developed in order to increase the yield and produce more limonene for use in industries and households.

CONCLUSION

From the study, it is evident that citrus peels contain limonene which can be successfully extracted through simple distillation technique. It was found that orange (*Citrus sinensis*) peels had more limonene yield than lemon (*Citrus limon*) peels and lime (*Citrus aurantifolia*) peels had the lowest limonene yield. Characterization of the extracts helped to prove that they were limonene as they showed positive results for terpenoid and unsaturation. They were also insoluble in water, neutral in nature and had characteristic fruity aroma depending on the source.

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