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Extraction and Isolation of Limonene and Formation of Pectin from Citrus Fruits; Preparation of Citric acid from Lemon Juice and it's Derivative

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Abstract: *Essential oils are highly concentrated substances extracted from flowers, for their flavour and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Extraction of essential oils is one of the most time- and effort consuming processes. The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties. Unfortunately, many of the solvents are used in industry and retail are volatile organic compounds (VOCs) which leads to environmental damage, through pollution, risk to human health and to resource depletion, we need to develop and apply more environmentally friendly approaches. So all traditional and old extraction routes obviously give adverse effects to the mankind and all living beings. There are wide number of ways to extract the Essential oil but the quality never remains the same. Here we are using the "Steam Distillation" method for extraction which is the cheapest way for the extraction of Oils from the different parts of the plants.*

This research describes the extraction of Citrus oil. It is an essential oil present within the rind of wall of a citrus fruit. In contrast to most essential oils, it is extracted as a by-product of juice extraction by "Steam Distillation". In the present investigation orange (citrus sinensis) peels is used for the extraction of citrus oil. The citrus oil composed of around 95% D- limonene which has many applications ranging from food flavouring agents to cosmetics..

Keywords: Citrus oil, Orange peels, essential oil, Steam distillation

1. Introduction

An essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, or simply as the oil of the plant from which they were extracted, such as citrus oil. An essential oil gets its name from the plants from which it is derived. These oils were given the name "Essential" because they were believed to capture a plant's essence,that is its odour and flavour. They lend plants their distinctive fragrance. Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe.

Essential oils are generally derived from one or more plant parts, such as fruits (bergamot, orange, lemon, juniper Citrus fruits belong to six genera further commercial form. Peel of citrus fruit has numerous glands that contain oil that is typically recovered as major by product. Each citrus fruit has its own characteristic set of compounds that comprise

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the oil and that are responsible for its flavour and aroma to products such as carbonated drinks, ice-creams, cakes, air-fresheners and perfumes.

D-Limonene shown in fig. 1. is a common naturally occurring compound with a citrus scent. It is often used as an additive in food products and fragrances, and is classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS). It has also been approved by the U.S. Environmental Protection Agency (EPA) for usage as a natural pesticide and insect repellent.

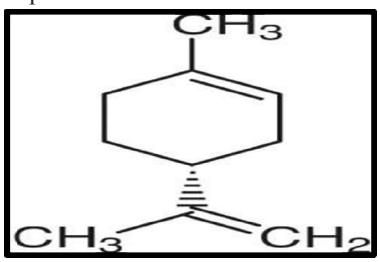

Figure 1. Molecular structure of limonene.

Limonene has also been studied for its anti-carcinogenic properties. Orange oil, which contains a considerable amount of limonene, has numerous applications including a combustion in engines, a powerful degreaser in cleaning applications, and a natural pesticide. These uses may require a known concentration of limonene with a limited amount of impurities. This exemplifies the need for a reliable method of extraction of limonene from its natural source, citrus rinds, followed by a quantitative analysis of the extract for limonene and possible impurities.

The main objective of this work is to extract the optimum amount of citrus oil from orange peels by steam distillation and also to Vermicompost the orange peels residue left over after the oil extraction. In this research, solvent extraction, steam distillation, and water distillation methods use to extract oil from orange peel and compare those terms of costing, yielding and time and reproducing better natural aroma of orange essential oil.

Essential oils can be extracted using various methods. Process choice significantly impacts yield and quality, leading to the development of processes aiming for maximum essential oil (EO) yields in a chemical state close to their native structure. In this chapter, various extraction techniques, including conventional ones and their intensification, are discussed along with their respective pros and cons. Additionally, new eco-friendly extraction methods have been introduced to enhance the conventional production of essential oils.

The most traditional, straightforward, and widely utilized extraction techniques are hydro-distillation and steam extraction. In actuality, steam extraction techniques are used to extract 93% of all essential oils. Study on extraction of oil by hydro distillation and solvent extraction methods using different solvents like water, n hexane, methanol, petroleum ether Were done. The experimental results obtained from the extraction and the characterization of the essential oil from orange peels have shown that the maximum yield of essential oil obtained from the orange peels used in this work were 3 % and 2 % when the methods employed were hydro distillation and solvent extraction respectively, indicating that water distillation was able to give the highest yield of essential oil among the methods considered.

LIMONENE

Limonene is the most important Monoterpenoid which is widely distributed in nature. Its R (+) Limonene form occurs in Lemon and Orange oils & its S(-)Limonene form occurs in peppermint oil whereas the (±) form occurs in turpentine oil. The racemic modification of limonene is known as Dipentene. This name was given to inactive form before its relation to the active form (Limonene) was established. Limonene is produced by racemisation of the optically active forms at about 250^0C. Now a day they also use solvent extraction, water distillation and steam distillation methods. The basic parameters influencing the quality of an extract are the solvent used for extraction, the manufacturing process (extraction technology) used with the type of equipment employed. In this research, I have successfully extracted Limonene form citrus fruits rind by steam distillation method.

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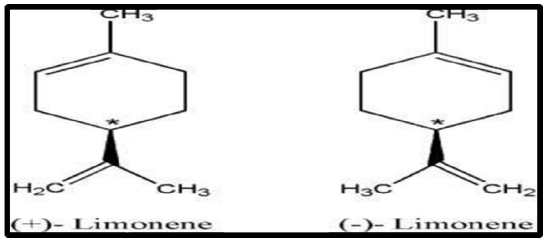

Figure 2: Structure of R-(+) limonene and S-(–) limonene

2. LITERATURE REVIEW

"Citrus essential oils are characterized by a volatile and a nonvolatile fraction, which can be composed of more than 200 compounds". The volatile fraction is mainly composed of monoterpene and hydrocarbons, also by their oxygenated derivatives, aliphatic aldehydes, alcohols and esters, forming until 99% of the essential oil. The non-volatile fraction could contain hydrocarbons, sterols, fatty acids, waxes, carotenoids, psoralens, and flavonoids.

Terpenes, the main components of essential oil, are produced by various species of plants and present diverse functions, such as defense mechanism against herbivores and pathogens and plant developmental physiology. The terpene basic chemical structure consists of an isoprene unit. Terpenes can present diverse chemical structure and they are synthesized by metabolic pathways by several types of specialized plant cells. Terpenes are employed in disease prevention and treatment, offering various biological effects such as antimicrobial, antiallergenic, antioxidant, anti-inflammatory and immunomodulatory properties. The terpenes large use in health can also be explained by its favourable pharmacokinetic properties, such as lipophilicity and low molecular weight.

Limonene is one of the most common terpenes in nature and a major constituent of numerous essential oils from Citrus. Limonene is a colourless liquid and it exists as two optical isomers, named d- or l limonene, and as a racemic mixture. Limonene possesses a pleasant lemon-like odour, which makes it widely used as a flavour and fragrance additive in common food items, such as fruit juices, candies, chewing gums, soft drinks, ice creams. Limonene is one of the most frequent and inexpensive fragrances used in cosmetics formulation, and can be found in many types of beauty products such as soaps, perfumes, shampoos, hair conditioners, and shower gels cleaning products and eco-friendly pesticides. In addition, it is considered safe for food preservation and could be used as a green solvent for the extraction of natural products. After oral administration, limonene is rapidly absorbed in the gastrointestinal tract, distributed and metabolized. Limonene is considered safe, presenting low toxicity to humans, without inducing mutagenic, carcinogenic, or nephrotoxic risk to humans. d-Limonene was first recovered as a commercial product during the 1941–42 Florida (USA) citrus season, from the steam evaporater condensate in the production of citrus molasses.

Schmidt and Göen investigated the limonene metabolism and elimination kinetics in humans. The authors found that the metabolites carveol, perillic acid, limonene-1,2-diol, and limonene-8,9-diol, but not perillyl alcohol. Human limonene metabolism occurs rapidly, and the body is almost entirely cleared from the metabolites after 24 h of limonene ingestion.

Numerous therapeutic properties have been attributed to limonene (1-methyl-4-(1-methylethenyl) cyclohexane), a naturally occurring 10-carbon cyclohexanoid monoterpene derivative. The present review article covers the last ten years (2008–2017) of publications about limonene therapeutic effects published in the scientific literature, aiming to disseminate the knowledge about this useful compound and shed light in future researches regarding its biological activities in preclinical and clinical studies.

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3. METHODOLOGY

EXTRACTION OF ESSENTIAL OIL (LIMONENE) FROM CITRUS FRUITS

Experimental Process :

Material Used :-

The materials used for this work were round bottom flask, distillation unit, thermometer, measuring cylinder, conical flask, separating funnel.

Sample Collection :-

The orange peels samples are collected from the local juice vendors

Samples of lemon, orange, and grapefruit rinds were carefully collected using a Grater.

Grate the outer orange coloured rind of oranges and add to 100 cm^3 of distilled water in the 250 cm^3 round bottom flask. Add small piece of porcelain. Heat the flask so that distillation proceeds at a steady rate, approximately one drop per second of distillate (Note : Take care not to let the liquid in the round bottomed flask boil too strongly).

Collect approximately 50 cm^3 of distillate in the measuring cylinder. The oil layer will be on the surface.

Using a pasture pipette carefully collect the oil layer into clean and dry container shown in fig. 6 for the next procedure.

Separation of Essential oil (Limonene)

This can be done using techniques such as Decantation or using a Separating funnel. We can use Separating funnel for the separation of essential oil. Take all the distillate in separating funnel and add 5ml chloroform shown in fig. 3. Shake the funnel slowly and allow to stand for 10 minutes. After that two layers will be separated which is oily and aqueous. Collect the upper layer (oily layer) in container and allow to evaporate the chloroform.

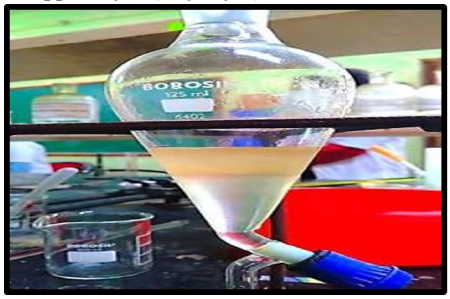

Figure. 3. Limonene separated by Separating Funnel

Figure. 4. Limonene Collect in container

ISOLATION OF LIMONENE

Weigh accurately about 1 g of the oil sample in 250 cm^3 conical flask. Add to it 5 cm^3 of toluene. Add 15 cm^3 of the hydroxylamine hydrochloride solution. Add one drop of methyl orange indicator and titrate with the 0.5 N alcoholic KOH solution, till the red colour changes to permanent yellow colour. Note down the CBR. Standardize the 0.5 N alcoholic KOH solution with succinic acid.

Calculation for aldehyde content in citrus oil : 17.65%

Results :

Volume of alcoholic KOH required = **2.5 cm^3**

Percentage of aldehyde in the given sample of oil is **17. 65 %.**

FORMATION OF PECTIN FROM RIND OF GRAPE FRUIT

Peel off the grape fruit rind. Cut the inner rind into small pieces. Weight the 25 gm of inner rind in 250 cm^3 beaker and add 100 ml of distilled water and Boil for few minutes. After that add 2 ml diluted acid which is HCL or H_2SO_4 and stir continuously to distribute acid well. Cover the lid to speed up boiling. Now add Ethanol in the centre of beaker in order to form gel, pour it fast otherwise immediately gel surface is formed. Filter it with a clean and pre wetted muslin cloth. Repeat the procedure 2 to 3 times or more to get maximum yield.

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Figure.6 Pectin formation using HCL & Ethanol

4. RESULT AND DISCUSSION

Experimentally we extract the essential oil (Limonene) by steam distillation method. Our results revealed that it was possible to produce value added products from the orange peel wastes such as essential oil.

Physical Form	Liquid
Molecular Formula	$C_{10}H_{16}$
Colour	Colourless to pale yellow
Boiling Point	176^0C
Solubility	Insoluble in water
RF Value	0.9

Citric acid is an organic compound with the chemical formula $C_6H_8O_7$. It is a colourless weak organic acid. It occurs naturally in citrus fruits. In biochemistry, it is intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms.

Citric acid is an important natural compound that has been known since the late 18^{th} century. The pioneering SwedishGerman chemist ***"Carl Wilhelm Scheele"*** isolated it from lemon juice in 1784. It has since been found in other citrus fruits.

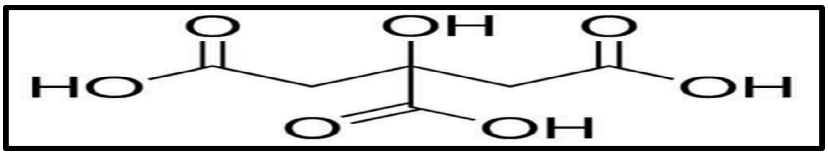

Figure.7 Structure of Citric Acid

EXTRACTION OF CITRIC ACID FROM LEMON JUICE : Squeezed the 8 lemons and collect the 20 ml (A) juice in 500 cm^3 beaker. Check the pH of juice which is 2-3 it is more acidic. Add 10% Sodium hydroxide solution in beaker till neutralized the juice. Filter the solution by coffee filter. Collect the filtrate in clean 500 cm^3 beaker. Add

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calcium chloride in beaker (28.5 g $CaCl_2$ to 70 ml water). Heat the flask on burner to form calcium citrate precipitate. Filter on vauccum filtration washing with hot water 3 times. In calcium citrate precipitate add sulfuric acid (20.9 ml 98% dissolved in 200 ml water). Stir thoroughly for few Minutes top form calcium sulphate. Calcium sulphate presentation washing with small amount of water and collect the filtrate and evaporate the water. Dry the crystals and weigh it.

Calculation:

Weight of crystals = **3.6 gm**

Percentage Yield of Product : 18%

Figure. 8 Extraction of citric acid form lemon juice

5. CONCLUSION

Limonene is an important natural compound with a wide range of uses. An impressive amount of work has been conducted on the biotransformation role in the production of other monoterpenes. The promising insecticidal activity of limonene provides a possible alternative natural insecticide for the control of pests in an attempt to prevent the spoilage of stored products. The environmental pollution arises due to the orange peels can overcome by using the same for citrus oil extraction by steam distillation. The optimum amount of citrus oil 2.4ml/100g of orange peels can be extracted by steam distillation at the optimum condition of temperature 96^0C time 60 min. and solid to solvent ratio 100g/200ml. Limonene has appreciably good chemopreventive activity which renders a great opportunity for further investigation. It is thus noteworthy stating that more interesting biological activities, biochemical modifications, as well as ecological findings, are likely to emanate from future research on this monoterpene hydrocarbon.

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It gives us immense pleasure to present my project on “Extraction & isolation of limonene and formation of pectin from citrus fruits; preparation of citric acid from lemon juice and it’s derivative”. The able guidance of all teaching staff of department made this study possible.

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