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Effects of Modifier Polarity on Extraction of Limonene from *Citrus Sinensis L. Osbeck* Using Supercritical Carbon Dioxide

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ABSTRACT

Limonene constitutes 98% of the essential oil obtained from orange peel. Besides being used as fragrances and flavours in the food, perfume and cosmetic industries, limonene is also a good degreasing agent. Supercritical carbon dioxide is an excellent solvent for non-polar compound like limonene but poor solvent for polar compound like α-terpineol. Common practice in supercritical fluid extraction is to change the polarity of supercritical carbon dioxide by employing polar modifiers to increase its solvating power towards polar analytes. Base on this, in the attempt to extract more limonene in orange essential oil, less polar modifiers were added instead. In this study, effects of adding modifiers with different polarity on extraction of aroma compounds (limonene, linalool and α-terpineol) from *Citrus Sinensis L. Osbeck* or sweet orange peel were investigated. Supercritical extraction was carried out at defined pressure and temperature for duration of 45 minutes. Concentration of aroma compounds extracted was analysed using GC-MS. The optimum conditions for extraction were observed at 318K and 12MPa. The concentrations of limonene increased significantly by the addition of methanol and slightly with n-heptane. It was also found that n-heptane is effective on supercritical CO_2 extractions of linalool and α-terpineol.

| Limonene | Supercritical carbon dioxide | Modifier | Extraction | Orange essential oil |

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1. INTRODUCTION

The processing of oranges produces a series of by-products. The peel is one of them, from which a variety of products including essential oil can be obtained [1]. It is usually extracted from citrus fruit peel by cold pressing or steam distillation. The compositions between different types of citrus also have been compared at different stages of maturity of the fruits [2]. The essential oils are widely used in variety of products such as food, beverage, pharmaceutical, perfumes and cosmetics industries. Besides the limited source of supply, the small amount of essential oils that are contained in each aromatic plant makes it even more valuable.

The genus *Citrus* includes several fruits such as oranges, mandarins, lime, lemons and grape fruits. *Citrus sinesis (L.) Osbeck* or also known as sweet orange is a hybrid of ancient cultivated origin, possibly between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulate*). The essential oil is present in fruit flavedo in abundant quantities. Citrus oil is the essential oil composed of two main components of which are the terpene hydrocarbons (major component) and oxygenated terpenoids. The citrus oil contains over 95% of terpenes, less than 5% oxygenated compounds and less than 1% non-volatiles such as wax and pigments [3]. Various antioxidant compounds were found in citrus oils including flavonoids, phenolic acids and terpenes, such as d-limonene [4]. Ten compounds were described as aromatic components in the orange peel oil (*Citrus sinesis (L.) Osbeck*) where linalool, α-pinene, and decanal presented highest aroma intensity [5]. Limonene or d-limonene is the major component of the oil extracted from citrus rind. Content of d-limonene varies with orange variety, manufacturing process of orange juice and extraction process. Another aromatic compound usually found in citrus essential oil is linalool, which has pleasant scent of floral with a touch of spiciness. The structure of limonene and linalool are illustrated in Figure 1.0 and Figure 2.0.

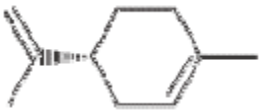

Fig. 1 Structure of limonene

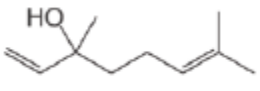

Fig. 2 Structure of linalool

Most papers published from 1979 to 1999 on the composition of sweet orange oils have been reviewed by

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Dugo *et. al.* [6]. From his review, limonene gives the highest percentage composition of the volatile fraction of sweet orange oils, be it cold-pressed oils, commercial and unusual oils or laboratory-extracted oils.

Methods used to extract essential oils today are based on the ancient principles of maceration, expression and steam distillation. Steam distillation has traditionally been applied to recover essential oils from plant materials however essential oils undergo chemical alteration and the heat-sensitive compounds are easily destroyed [7]. There are various types of solvent use in supercritical fluid extraction depending on the characteristics of component extract and properties of the solvent itself. Practically, more than 90% of all analytical supercritical fluid extraction is performed with carbon dioxide for several reasons. Besides having relatively low critical pressure and temperature, CO_2 is relatively non-toxic, non-flammable, odourless, colourless, absence of residual problems and inexpensive [3].

However, main disadvantage of CO_2 is its lack of polarity for the extraction of polar analytes [8]. Because of this, polar modifiers or co-solvents are introduced in the SFE system to aid extraction of polar analytes. Co-solvent will modify the solvent power or the selectivity of carbon dioxide as well as to enhance the affinity of the solvent mixture towards polar compounds. Li *et. al.* [9] studied on the co-solvent effects of ethanol, acetone and ethylene glycol and compared for benzamide.

Although few studies have been conducted to extract aroma compounds of citrus fruits in general and on orange peel in particular using supercritical fluid extraction [10], the effects of modifier polarity on extraction of polar and nonpolar aroma compound present in orange peel has not been well established. Hence, the aim of this research is to focus on the study of the effect of modifier with different polarity towards extraction of polar and non-polar aroma compounds extracted from *Citrus Sinesis L.* peel using supercritical carbon dioxide (S-CO_2) as solvent.

2. EXPERIMENTAL

2.1 Materials and chemicals

The sweet oranges used in this work were *Citrus Sinensis L. Osbeck* purchased from local supermarket. Only the external part of the oranges (flavedo) was peeled and the peel of fresh oranges was dried at 50^{o}C in dryer for 7 hours. After drying, the orange peel was placed in hermetically sealed bags and stored for two months in cold chamber at 4^{o}C until grinding was performed.

Dried orange peel was ground and sieved. The grinded samples were placed in hermetically sealed bags and stored in a cold chamber at 4^{o}C until extraction was performed. No more than 20 da ys should elapse between grinding and extraction [10].

The CO_2 gas of more than 99% purity was used. The limonene, linalool and α-terpineol standard was purchased from Merck. The methanol, dichloromethane and n-heptane, all of HPLC grade, used as modifier were purchased from Qrec.

2.2 Supercritical fluid extraction method

The extractions were performed using SFT-150 Supercritical Fluid Extraction system (Supercritical Fluid Technologies Inc. DE, Newark). For each experiment, 10g of grinded orange peel was packed in the extraction vessel. The filled vessel was then inserted into the thermal-controlled extraction chamber. Liquified supercritical CO_2 was pumped into the sample vessel. Both the temperature and pressure of the vessel will be automatically reached and maintained by a control unit according to settings. The flowrate of CO_2 was regulated by both the pressure releasing valve and a thermal-control restrictor and monitored by a flow meter. Extracts were collected in glass tubes at ambient temperature and atmospheric pressure.

To study the influence of pressure and temperature on essential oil yield, a series of experiment was designed to be performed in 8 MPa and 12 MPa pressure and 35^{o}C and 45^{o}C temperature. In all experiments, temperature applied was lower than 50^{o}C, which is the temperature employed in drying the orange peel. This is because thermal degradation could occur at higher temperatures.

To study the effect of modifiers added to the solvent, two different modifiers with different polarity are use. The extraction was carried out at 35^{o}C and 12.5 MPa. According to study done by Mira *et. al.* [10], the optimum conditions for limonene extraction were 12.5MPa and 308K, where in these conditions limonene represents more than 99.5% of the orange peel essential oil. Experiment was first carried out with pure supercritical carbon dioxide, without the use of modifier, followed by methanol and n-heptane as modifiers. Due to the sudden malfunction of SFE equipment, effects of using dichloromethane as modifier as well as effects of modifiers concentration on extraction of polar and non-polar compounds could not be carried out but will be considered in my succeeding study. Each extraction was performed for duration of 45 minutes and the extract was collected inside glass tube containing 10ml methanol as trap solvent. The extracts were kept at 4^{o}C until analysis was performed.

2.3 Analysis method

The analysis of the standards and samples extracted was performed with a Perkin Elmer Clarus 560 D GC/MS system. The column used throughout was a Perkin Elmer Elite-5ms (30m x 0.25 mm i.d. x 0.25 μm film thickness) column. Helium was used as a gas carrier with the flow rate of 1mL/min. The injector temperature was maintained at 250^{o}C. The GC was fitted with a capillary injector port using a 4-mm standard glass liner packed with quartz wool configured for split operation. Samples of 0.5 μL were injected in split mode (20 mL/min). The oven temperature was held at 80^{o}C for 3 min at 5^{o}C/min, raised to 140^{o}C and

then 275°C at 45°C/min. Libraries of computerized spectral databases were used to compare both masses and fragments and their intensities. Once likely match was found, the components were identified and quantitative analysis was carried out.

3. RESULTS & DISCUSSION

3.1 Essential oil yield

The yield of orange peel oil ranges from 0.2 to 0.5%, depending on the variety, production area, and harvesting season of the fruit, as well as the oil production method. Terpene hydrocarbon, mainly d-limonene made up the major components of the peel oil which is more than 90%. The other main components are oxygen compounds, such as linalool, aldehydes and esters. Linalool decreases with fruit ripening, whereas aldehydes and esters increase [11].

Figure 3.0 and 4.0 summarize the results of percentage yield of essential oil for extraction at two different pressures and two different temperatures. For each pressure and temperature, methanol and dichloromethane with two concentrations were used. Percentages of yielded essential oil are calculated using equation below.

$$\text{oil yield \% amount essential} = \frac{\text{weight of essential oil collected}}{\text{weight of sample}}$$

The result shows that extraction at 12 MPa pressure with the temperature of 318K gave the highest yield followed by extraction at 12 MPa pressure and 308K. The average lowest yield obtained is when the extraction done at 8 MPa pressure with 308K temperature. Meanwhile, for extraction with pure carbon dioxide without co-solvent, the least yield was obtained. Higher concentrations of modifier gave higher percentage of essential oil yield.

The solvating power of supercritical fluids can be manipulated by changing pressure and temperature and by doing so, a r emarkably high selectivity can be achieved [12]. In his work of studying the effect of adding methanol, water and dimethyl sulphoxide as modifiers, Casas *et. al.* [12] shows that, at constant temperature, raising the pressure increases the density of supercritical fluid i.e. its solvating power becomes greater and more substances are transferred to the supercritical CO_2, meaning that the extraction process is favoured. Increasing temperature at constant pressure of 100bar, however, cause a decrease in the extraction yield which is attributed to the decrease in the density of the supercritical fluid. Nevertheless, at higher pressure of 500bar, an increase in the temperature benefits the extraction process due to the increase in the vapour pressure of the substances extracted.

The fluid pressure is the main parameter that influences the extraction efficiency. An elevation of this pressure at a given temperature results in an increase in the fluid density, which means an enhanced solubility of the solutes. Therefore, the higher the extraction pressure, less volume of fluid is necessary for a given extraction [13]. Pourmortazavi *et al*. [13] further suggested that at constant pressure, the density of CO_2 decreases when the temperature is increased. This effect becomes more pronounced as the fluid compressibility increases. However, temperature also affects the volatility of the solute. Thus, the effect of raise in temperature is difficult to predict because of its dependence on the nature of the sample. For a volatile solute such as aroma compounds, there is a competition between its solubility in CO_2 which decreases as temperature increases, and its volatility which rises with increasing temperature.

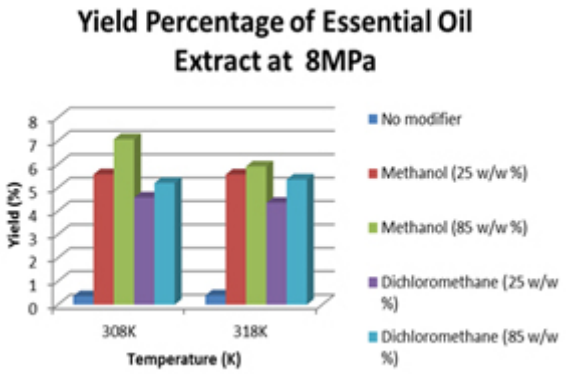

Fig. 3 Percentage of yield of essential oil at 8MPa

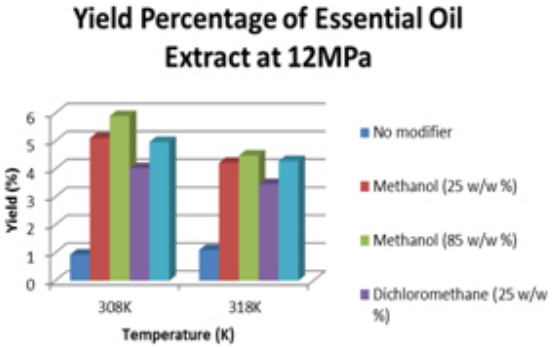

Fig. 4 Percentage of yield of essential oil at 12MPa

3.2 Effect of different modifiers in essential oils extraction

Each essential oil extracts were analyzed using GC-MS. Each compound in the samples eluted from the column will give peaks in the chromatogram as shown in Figure 5.0, 6.0 and 7.0.

Figure 5.0, 6.0 and 7.0 shows the GC-MS ion chromatogram of compounds identified in orange peel extract by extraction with supercritical CO_2 without modifier, with addition of methanol and with addition of n-heptane respectively. In the extracts, among compounds identified include β–myrcene, d-limonene, β-pinene, linalool and α–terpineol. The limonene was the principal

compound extracted in all essential oil samples. Study by Tirado *et. al.* [2] also found out that limonene was the main component in citrus fruit extract which is 91.03 – 92.57%.

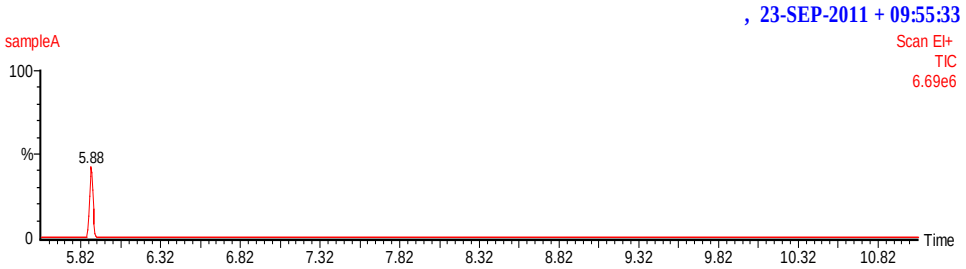

Fig. 5 The extracted ion chromatogram for the essential oil extracted with pure supercritical CO_2

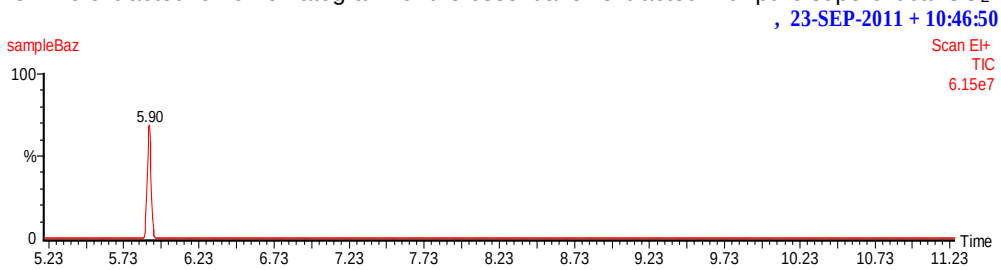

Fig. 6 The extracted ion chromatogram for the essential oil extracted with supercritical CO_2 and methanol

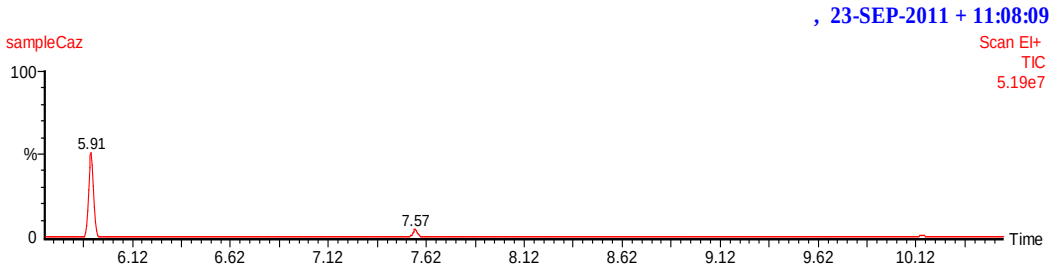

Fig. 7 The extracted ion chromatogram for the essential oil extracted with supercritical CO_2 and n-heptane

The nature of the modifier depends on the nature of the nature of the solute to be extracted. Modifier exerts its effect by interacting with the analyte/matrix complex to promote rapid desorption into the supercritical fluid, and by enhancing the solubility properties of supercritical CO_2 [14]. According to Casas *et. al.* [12], depending on the type of sample matrix and the affinity of the analyte for the matrix, the modifier may influence the extraction in three different ways: (1) by increasing the analyte's solubility in the supercritical fluid as consequence of analyte-modifier interactions in the fluid phase; (2) by facilitating analyte desorption, where the molecules of polar modifiers are able to interact with the matrix and compete efficiently with the analyte for the active sites in the matrix; (3) by distorting the matrix-analyte diffusion process and favour penetration of the supercritical fluid inside the matrix when the modifier swells the matrix. Yang *et. al.* [15] demonstrated that combination of co-solvent with high temperature is highly effective. Most SFE applications use methanol as modifier, but in some cases, other co-solvents such as hexane, aniline, toluene, or diethylamine have been shown to be more efficient [15,16].

Table 1.0 below summarizes the concentration of limonene, linalool and alpha terpineol presence in the orange peel extract. Highest concentration of limonene was obtained in the extract extracted using supercritical CO_2 and addition of methanol as modifier, followed by extract extracted using supercritical CO_2 and addition of n-heptane as modifier. Linalool and α-terpineol were only detected in extract extracted using supercritical CO_2 and n-heptane. Methanol is polar in nature, however addition of methanol as modifier also increase the concentration of limonene which is less polar compound compared to using n-heptane (non-polar) as modifier. When n-heptane is added to the SFE system, it enhanced the extraction of linalool as well as α-terpineol, which is more polar in nature.

Shimoyama *et. al.* [17] studied the effects of co-solvents such as acetone, hexane, water and ethanol on solubilities of 1,8-cineole and α-pinene in supercritical CO_2. The solubilities of α-pinene in supercritical CO_2 with ethanol are slightly higher than those without co-solvent. He

also observed that solubilities of α-pinene in supercritical CO_2 are remarkably reduced when using hexane as modifier. He suggested that this is caused from the strong interactions between α-pinene and hexane molecules in liquid phase because both α-pinene and hexane are non-polar hydrocarbons. This could be the same reason which cause decrease in concentration of limonene obtained from extraction using n-heptane as modifier. Both limonene and n-heptane is non-polar in nature.

Table 1 Concentration of limonene, linalool and α–terpineol identified in orange peel extract

Compound	Area percent		
	Without modifier (pure S-CO_2)	S-CO_2 + methanol	S-CO_2 + n-heptane
d-limonene	10.547	22.316	10.722
linalool	-	-	0.865
α- terpineol	-	-	0.159

Pourmortazavi *et. al*. [18] studied the supercritical fluid extraction of aerial parts of *P. atriplicifolia*. The study evaluated the effect of different modifiers at a co nstant pressure and temperature on the extraction efficiency. The results of the study showed that changing modifier type and identity could significantly affect the selectivity of the extraction process. In the presence of hexane as modifier, the percent of α-pinene, β-pinene, δ-3-carene, α-terpenyl acetate increased whereas the percent of 1.8-cineole + limonene and camphor decreased in comparison with extraction by pure CO_2.

3.3. Factors that effects essential oil extraction

In this study, there are also some other factors which can be considered that might affect the produced essential oil. At the beginning of study, sample preparation should be done properly to protect the samples from contamination. Then, the size and surface area of the sample will also affect the essential oil production. Decreasing the particle size of solid matrices leads to higher surface area, making extraction more efficient. Nevertheless, excessive grinding may obstruct the extraction due to re-adsorption of the analytes onto matrix surfaces and pressure drop inside the extraction chamber [13]. Orange peel samples should be dried however, samples prepared for this study might not have been dried completely. According to Casas *et. al.* [12], the best extraction yields were obtained from dried samples. Moisture from the congealed samples seems to be a factor that diminishes the extraction yield, with the water acting as a solvent that competes with supercritical CO_2. Excess water remaining in the extraction vessel will cause the highly water-soluble solutes to partition into the aqueous phase and therefore SFE recovery will be low.

The modifier can either be added to the sample in the extraction cell prior to supercritical fluid equipment or be mixed with the CO_2. The latter is more effective since the modifier is continuously passed through the sample [14]. In this study, the first method was used where the modifier was swept from the extraction cell when the supercritical fluid starts to circulate through the sample and this might has affected the accuracy of the extraction.

4. CONCLUSION

The solvent power of supercritical CO_2 is smaller compared to those of liquid solvents. Co-solvent or modifiers are often added in supercritical CO_2 extraction in order to increase the solvating power or the selectivity in supercritical CO_2 in extraction of highly polar analytes. The purpose of this study is to look at the possibility to increase the extraction of limonene which is non-polar by adding n-heptane as modifier to supercritical CO_2.

Supercritical carbon dioxide was used to extract essential oil from sweet orange peel. Effects of pressure and temperature as well as addition of modifiers with different polarity were investigated. The optimum conditions for extraction were at 318K and 12 MPa. The concentrations of limonene increased significantly by the addition of methanol and slightly with n-heptane. Concentration of limonene decreases by the addition of n-heptane since they are both having low polarity in nature. However, it is found that n-heptane is effective on supercritical CO_2 extractions of linalool and α-terpineol. Subsequent study will be done to look at the effect of more type of modifier with different polarity at different concentrations on the extraction efficiency of polar and non-polar compounds from sweet orange peel essential oils.

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