# Separation of *Agathosma* (Buchu) Essential Oil Components: A Phase Equilibria Study for the Feasibility of Isolation of Components Using Supercritical Carbon Dioxide

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## ABSTRACT

The essential oils of the *Agathosma* genus are potential sources of a variety of valuable compounds. Of particular interest is the isolation of sulphur terpenoids, which are important flavour compounds. The removal of terpenes, which are prone to oxidation, and tend to reduce the shelf life of the oil, is also important. Thermal methods of fractionating essential oils denature thermally fragile molecules and solvent extraction using organic solvents leaves traces of potentially harmful solvents in the product. Supercritical fluid fractionation is therefore an attractive alternative.

This paper presents results on the phase behaviour of binary systems of pulegone, eucalyptol, limonene, diosphenol, p-menth-3-one-8-thioacetate and terpenen-4-ol in supercritical $CO_2$. Bubble- and dew-point pressures at temperatures between 308 K and 358 K and heavy component mass fractions between 0.65 and 0.013 were measured using a high pressure, synthetic, static view cell. It was found that at 308 K the transition pressures of all compounds were virtually similar, varying between 6 MPa and 8 MPa. At 338 K limonene and eucalyptol exhibited similar phase behaviour, with a transition pressure 3 MPa lower than the other compounds at the lower mass fractions. At 358 K, however, the transition pressure of p-menth-3-one-8-thioacetate was at least 3 MPa higher than the other compounds, yet still close to that of pulegone in the lower mass fractions. The strong influence of temperature on the solubility of the compounds in supercritical $CO_2$ indicates that selecting the correct temperature will probably result in separation of most compounds. However, the results indicate that separation of p-menth-3-one-8-thioacetate from pulegone may be difficult in the range of conditions investigated.

## INTRODUCTION

The *Agathosma* genus is indigenous to a narrow belt of distinct vegetation along the Southern tip of Africa, known as the fynbos. More than a hundred different species grow wild in this region, yet two, *A. betulina* and *A. crenulata*, locally known as buchu, are grown commercially. Buchu has been used traditionally by the indigenous Khoi and San people as a herbal remedy for various ailments, topically as well as internally. Its use as an analgesic,

antibacterial, antipyretic and diuretic has been shown scientifically [1]. In modern times, buchu is valued commercially as a source for the unique compounds found in its essential oil, used as flavour enhancers and in perfumes.

The aroma content in essential oils is made up mainly of the oxygenated terpenes, and sometimes sulphur terpenoids. Typical buchu essential oil is composed of 25 – 30 % hydrocarbon monoterpenes, 60 - 70 % oxygenated terpenes, and sulphur terpenoids. Coumarines, flavonoides and many other classes compounds have also been identified [1]. Typical terpenes found in buchu include limonene, beta-myrcene, sabinene, alpha-pinene and trans-beta-ocimene. The oxygenated terpenes include terpinolene, iso-menthone, diosphenole (buchu camphor) and eucalyptol. Terpenes in essential oils tend to oxidise on exposure to air, often resulting in offensive odours [2]. The sulphur terpenoids *cis/trans*-8-mercapto-*p*-menthan-3-one and *cis/trans*-8-acetylthio-*p*-menthan-3-one have been identified as the source for the blackcurrant, or cassis, flavour found in buchu essential oil [3]. Fractionation of buchu essential oil is therefore interesting for two reasons: the deterpenation of the oil gives it a longer shelf life, and concentration of the sulphur terpenoids increases its value.

Currently, vacuum distillation is used for separation of buchu oil components. The main draw-back with this process is the high process temperature which results in the decomposition of thermally labile components. The product is therefore not only denatured, but also expensive, due to low yields of valuable products, as well as the high energy requirements that are typical of distillation processes.

The advantages offered by supercritical fluid fractionation using carbon dioxide are therefore attractive. The use of a benign solvent not only conforms to the demands of today's consumer, but also obviates the need to remove the solvent by distillation. Low temperature processing preserves the chemical make-up of the oil.

In order to predict the suitability of a solvent such as carbon dioxide to selectively dissolve, and thereby separate, components of a mixture such as an essential oil, the phase behaviour of binary mixtures of the compound of interest with supercritical carbon dioxide are used. At a given temperature, significant differences in transition pressures between two binary systems indicate a preference of the solvent to dissolve the compound bearing the lower transition pressure. The solvent can therefore be used to separate the two compounds. High pressure vapour liquid equilibria data is therefore necessary to predict both the feasibility of separation and the possible operating conditions [4, 5].

Although the phase behaviour of binary mixtures of a variety of terpenes and their derivatives in supercritical carbon dioxide have been studied [6-8], the phase equilibrium data for most of the components of buchu essential oil in carbon dioxide could not be found in the open literature. For those that could be found, significant discrepancies were found between different sources [9, 10]. This paper reports the results of the measurements of the phase behaviour of binary mixtures of pulegone, eucalyptol, limonene, diosphenol, p-menth-3-one-8-thioacetate and terpenen-4-ol with supercritical carbon dioxide. This data enable the prediction of the feasibility of separation of the components, as well as to do a first order approximation of the suitable process conditions for separation.

## MATERIALS AND METHODS

A high pressure, variable volume view cell, previously designed and fabricated in-house, was used. The volume of the cell was 80 cm$^3$, with a capacity to operate up to 28 MPa and 460 K. The apparatus is described in detail elsewhere [11].

The maximum error of the mass fraction is estimated based on the accuracy of the balances used to measure the components loaded into the cell. This was estimated at 1% of the value. The accuracy of the temperature measurement is better than 0.2 K. The accuracy in the phase transition pressure measurement is 0.1 MPa.

The experimental procedure is described in detail in [11, 12]. It can be summarised as follows: A known amount of solute was loaded into the cell, after which the cell was evacuated by vacuum and flashed four times with carbon dioxide. A known mass of solvent, carbon dioxide, was then loaded before sealing the cell. When the cell reached thermal equilibrium the pressure was increased until a single phase mixture was achieved. The transition pressure was identified as the pressure at which the bubbles, or the first sign of vapour, appeared in the cell. Proper mixing was ensured with the use of a magnetic stirrer. Readings of the pressure, temperature and cell volume were logged. This process was repeated at ten degree temperature intervals in the temperature and composition range of measurement.

The materials used are shown in Table 1 below. The structures of the compounds are depicted in Figure 1.

**Table 1: The source, purity and CAS numbers of compounds used**

| Compound | Source | Purity | CAS |
|---|---|---|---|
| (R)-(+) d-Limonene | Sigma-Aldrich | 99% | 94293-57-9 |
| Eucalyptol | Sigma-Aldrich | 99% | 470-82-6 |
| Terpenen-4 ol | Penta | 99% | 562-74-3 |
| Pulegone | Sigma-Aldrich | 97% | 89-82-7 |
| Diosphenol | Penta | 98% | 490-03-9 |
| p-menth-3-one-8-thioacetate | Penta | 98% | 94293-57-9 |

(a) limonene     (b) eucalyptol     (c) 4-terpinenol

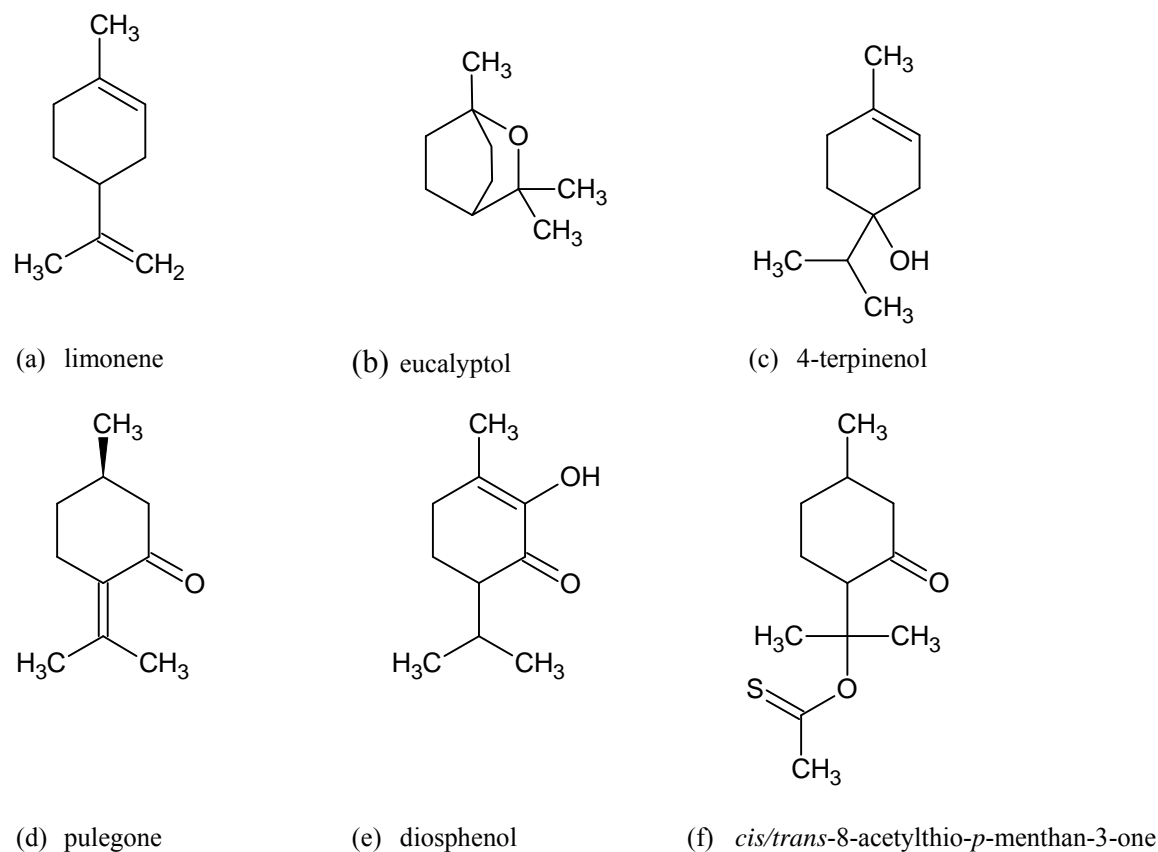(d) pulegone     (e) diosphenol     (f) *cis/trans*-8-acetylthio-*p*-menthan-3-one

**Figure 1: Structures of components used**

## RESULTS

The phase behaviour measurements were conducted for pulegone, limonene, eucalyptol, terpenen-4-ol, p-menth-3-one-8-thioacetate and diosphenol, in the solute mass fraction range 0.013 to 0.65, and temperatures between 308 K and 358 K. Although these compounds represent only a handful of the more than one hundred compounds present in buchu essential oils, these compounds can constitute over 80 % of the oil by mass [13]. The results will therefore provide a good a good idea of the separation that can be achieved.

In order to correct for slight differences in the temperatures during measurement, caused by day to day environmental temperature changes, isothermal data could be obtained by regressing the pressure versus temperature data to obtain a continuous curve that relates the transition pressure to temperature. For the majority of cases, this relationship could be described by a straight line with a coefficient of determination, $R^2$, better than 0.985. This value usually resulted in the error in pressure to be less that 0.1 MPa. Where the error value was larger, a quadratic equation sufficed.

The phase measurement was validated by comparing measured data for the limonene – carbon dioxide binary system with literature data presented by Leeke et al. [8] and Iwai et al. [14] at 318 K [10], and by Matos et al. [15] at 313 K. This data are compared in Figure 2.

The largest difference between the data measured in this study and that published in the three sources was 2.33 %, 2.37 % and 4.05 % for Leeke et al., Iwai et al. and Matos et al. respectively. The method of measurement in all three literature sources was the analytical-isothermal method with sampling.

The experimental phase transition pressures observed for all the components in this composition and temperature range were from 5.92 to 20.43 MPa. The variation of transition pressures with composition are shown in Figures 3. Data for diosphenol only appears for 348 K and higher temperatures. This is due to the high melting point of 356 K for the compound. Melting point depression allowed measurements to be carried out at temperatures as low as 348 K.
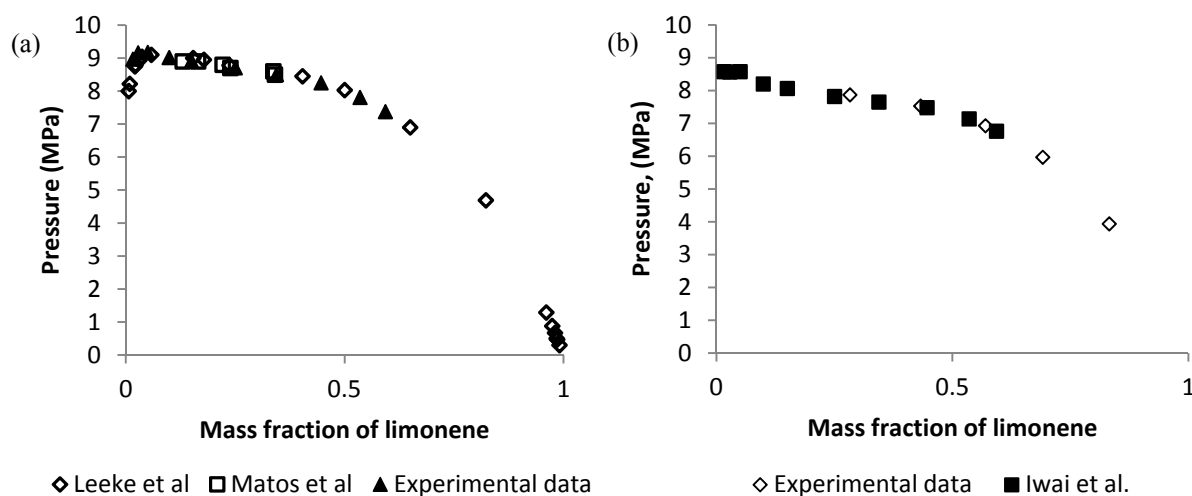


**Figure 2: Comparison of experimental data with literature data at (a) 318 K and (b) 313 K**

The measured data illustrate that an increase in temperature resulted in an increase in phase transition pressure, indicating a decrease in solubility, over the entire range of the investigation. Phase inversion, as well as evidence of three phase regions, was not observed over the entire range of measurement. The mixture critical points, located at the lower mass fractions at the lower temperatures, progressively moved towards the higher mass fractions as the temperature increased.

According to the experimental data, a clear demarcation appears at 338 K and higher temperatures. Limonene and eucalyptol are clearly more soluble than terpenen-4-ol, diosphenol, p-menth-3-one-8-thioacetate and pulegone. The segregation of the compounds confirms the known trend regarding solubility of natural compounds [16], illustrated in Figure 4.
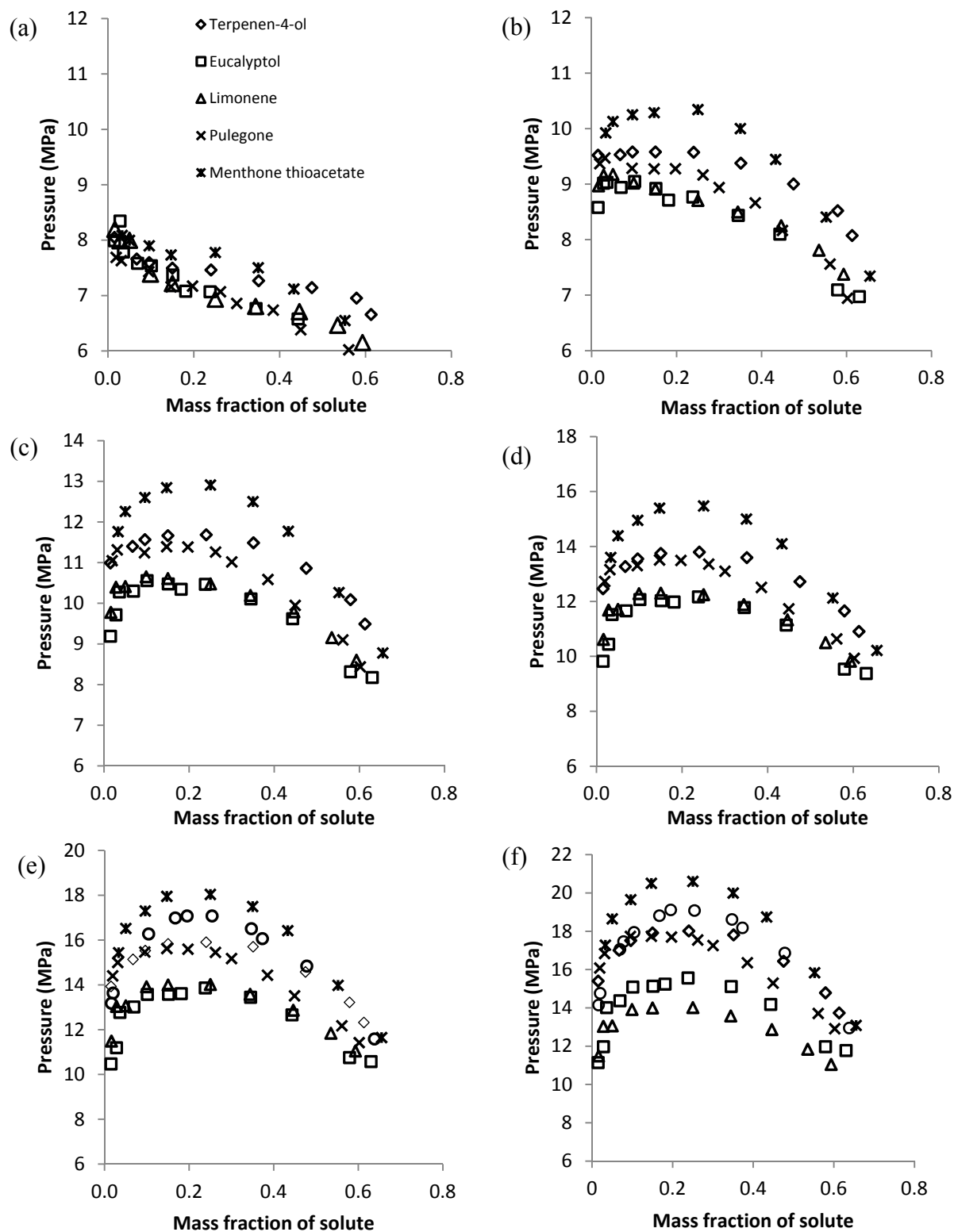
**Figure 3**: **Pressure- composition plots of buchu essential oil components measured at (a) 308 K, (b) 318 K, (c) 328 K, (d) 338 K, (e) 348 K, (f) 358 K**

*Decreasing solubility*

$\longrightarrow$

**Figure 4: Variation of solubility of essential oil components with type of compound - [13]**

Methyl thioacetate, pulegone and terpenen-4-ol are all substituted terpenes. Conforming to the above illustration they are expected to be less soluble than limonene. The anomaly observed in this data is the behaviour of eucalyptol, which is an oxygenated terpene, yet appears to be closer to limonene in its phase behaviour than to other oxygenated compounds. The structural differences between eucalyptol (Figure 1 (b)) and the other oxygenated terpines (Figure 1 (d) – (f)) can explain this anomaly. The presence of a single oxygen atom located in the ring, and the absence of a carbonyl group attached to the ring suggests that although eucalyptol is an oxygenated terpine, it is much less polar than the other compounds investigated. The polarity of solutes significantly affects their solubility in supercritical carbon dioxide [5].

The data shows that the difference in transition pressures of the terpenes and p-menth-3-one-8-thioacetate at 338 K is larger than 3 MPa. This difference increases to 6 MPa at 358 K. On the basis of this data, it can be predicted that the concentration of sulphur terpenoids by deterpenation using supercritical carbon dioxide is feasible. The data also shows that slight differences in process temperature can produce a different profile of compounds in the top or bottom products.  It is clear that the bottom product will likely be a mixture of substituted terpenes. Due to the similarity of solubilities of these compounds, especially in the low solute concentration region, separation of pulegone from this mixture using supercritical carbon dioxide may not easily be achieved in a single step. While higher temperatures favour better separation, lower temperatures reduce the risk of thermal degradation, and consume less energy. A careful selection of process conditions is therefore required.

## CONCLUSION

The phase behaviour of binary mixtures of the major components of buchu essential oil, pulegone, limonene, eucalyptol, terpenen-4-ol, p-menth-3-one-8-thioacetate and diosphenol, with supercritical carbon dioxide at 308 K to 358 K and 0.013 to 0.65 mass fraction was measured. No three phase regions or temperature inversions were observed in this range of conditions.

The results showed that there is a significant difference in the phase behaviour of the mono-terpene hydrocarbon limonene, and the substituted terpenes pulegone, eucalyptol, terpenen-4-ol, p-menth-3-one-8-thioacetate and diosphenol. This difference was most noticable at temperatures above 338 K. This indicated that above this temperature, supercritical carbon dioxide can be used to concentrate the substituted terpenes in buchu essential oil by removing the terpene fraction. However, the data also showed that, due to the similarity of phase

behaviour of the sulphur-type and the oxygenated terpenoids, the separation of pulegone from the sulphur terpenoids will be more difficult to achieve using supercritical carbon dioxide.

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