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Supercritical fractionation of *Agathosma* (buchu) essential oil. Part I: Measurement of binary phase equilibria

T.F.N. Madzimbamuto[a,b], C.E. Schwarz[a], J.H. Knoetze[a,*]

[a] Department of Process Engineering, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Department of Chemical Engineering, Cape Peninsula University of Technology, P.O. Box 1906, Bellville 7535, South Africa

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ABSTRACT

The value of essential oils can be improved by removing the potentially unstable compounds, such as monoterpene hydrocarbons (MT), in a process known as deterpenation. In order to determine the feasibility of deterpenation using supercritical CO_2, the phase behaviour of the pure components in CO_2 must be known. Phase equilibria data of most components found in buchu essential oil in supercritical CO_2 are not available in the literature. In this study, the phase equilibria of binary systems pulegone + CO_2, diosphenol + CO_2, terpinen-4-ol + CO_2 and limonene + CO_2 were measured between 308 K and 358 K, and at solute mass fractions between 0.0147 and 0.638, using a static synthetic view cell. The data were modelled using Chrastil's equation and separation factors determined. Oxygenated terpenes (OT) are significantly less soluble than MT, especially at the higher temperatures, indicating the possibility of separation.

1. Introduction

Agathosma is a genus of a flowering shrub that is indigenous to a narrow belt of distinct vegetation along the southern tip of Africa, known as the fynbos. More than a hundred and fifty different species are indigenous to this region [1], and of these only *Agathosma betulina* and *Agathosma crenulata*, locally known as buchu, are grown commercially. Buchu has traditionally been used topically as well as internally by the indigenous Khoi and San people as a herbal remedy for various ailments. Its efficacy as in analgesic, antibacterial, antipyretic and diuretic applications have been confirmed scientifically [2]. Presently, buchu is also valued commercially as a source for the unique compounds found in its essential oil, used as flavour enhancers [3] and in perfumes and colognes [2].

Only one variety of the buchu plant, *A. betulina*, is valued as a source of the flavour [3]. Oil of the *A. crenulata* variety, though it typically contains much higher levels of the valued sulphur terpenoids, also contains a high level of pulegone (50–60% in *A. crenulata* compared to less than 1% in *A. betulina*). Besides being toxic to humans at high concentrations [1], pulegone has a strong peppermint smell, which tends to mask the valued blackcurrant aroma [4]. However, the *A. crenulata* is the more hardy variety, surviving across a much wider area of the Cape region of South Africa. Pulegone from the oil of *A. crenulata* therefore may add value to the oil. Additionally, pulegone is also valued for use in perfumery and in aromatherapy.

The aroma content of many essential oils is made up mainly of the oxygenated monoterpenes (MT), and sometimes sulphur terpenoids (ST). Table 1 shows the results of a GC analysis of samples of *A. betulina* and *A. crenulata* essential oils. The composition is given on the basis of percent of peak area [5]. The oils are composed of about 15–20% hydrocarbon MT, 75% oxygenated monoterpenes (OT), up to 6% ST and numerous minor compounds that constitute less than 1% each. Other compounds not included in this table are present at compositions less than 1% in buchu essential oil. The MT include limonene and beta-myrcene while OT include pulegone, diosphenol (buchu camphor), terpinen-4-ol, iso-menthone and eucalyptol [5]. 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 of the blackcurrant (or cassis) flavour found in buchu essential oil [3,4]. On the other hand, since the hydrocarbon MT in essential oils tend to oxidise on exposure to air resulting in offensive odours [6], the processing of the essential oils thus entails both the removal of the terpene fraction and the concentration of the valuable compounds all in an environment that limits oxidation.

Currently, vacuum distillation and liquid–liquid extraction are the commonly used technologies for separating essential oil

* Corresponding author.
E-mail address: jhk@sun.ac.za (J.H. Knoetze).

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Nomenclature

Symbols

A	constant in the Chrastil equation
B	constant in the Chrastil equation
k	association number of molecules in a complex in the Chrastil equation
K_i	equilibrium constant for component i
N	number of data points
S_s	solubility of a solid in a SCF
SF_{ij}	separation factor of component i relative to component j
x_{CO_2}	mole fraction CO_2 in the liquid phase
x_i	mole fraction of component i in the liquid phase
y_i	mole fraction of component i in the vapour/supercritical phase
y_S	mole fraction solute in the SCF phase
ρ_{CO_2}	density of CO_2 at the solubility pressure and temperature
ρ_{SCF}	density of the SCF at the solubility pressure and temperature

Abbreviations

MT	monoterpenes
OT	oxygenated terpenes
ST	sulphur terpenoids
SC	supercritical
SCF	supercritical fluid
SCFF	supercritical fluid fractionation

components in industry. Although vacuum distillation has the advantage of short periods of exposure of the feed to high temperatures, evaporator temperatures can reach as high as 200 °C [6]. Thermally labile compounds tend to degrade at these temperatures. Solvent extraction has the disadvantages of requiring a further distillation step to remove the solvent. Traces of residual solvent invariably remain in the product.

Supercritical fluid fractionation (SCFF) has been shown to be a technically viable technology for the separation of essential oils. The feasibility of separation of hydrocarbon MT from OT in citrus oil has been widely reported [7,8]. Other examples of SCFF processes investigated in the literature include the isolation of sesquiterpenes from citrus oil [9], the production of novel varieties of hop aromas [10] and the isolation of capsaicin from capsicum oleoresin [11]. A review of industrial applications of supercritical fluids (SCF) is given by Knez [12]. SCF processing has also been used in combination with other separation techniques, such as simulated moving bed chromatography [13] and solid phase extraction [10]. The use of a benign solvent such as CO_2 not only conforms to the health demands of consumers, but also obviates the need to subsequently remove the solvent by distillation. In addition, low temperature processing preserves the chemical make-up of the oil and limits oxidation. The advantages offered by SCFF using CO_2 are therefore attractive.

In order to perform a preliminary feasibility study of the suitability of CO_2 as a solvent to separate compounds in a mixture, the solubilities of the respective pure compounds in the SCF can be compared. At a given temperature, a significant difference in the solubility indicates that separation in a counter-current column may be possible. Measuring the phase behaviour of different compositions of binary solute + CO_2 mixtures at different temperatures is a convenient way to obtain their solubility in CO_2. One such measure of phase behaviour is the measurement of bubble and dew point pressures for different compositions of the binary mixtures, at different temperatures. When considering bubble and dew-point data, the higher the pressure needed to transcend from a two phase to a single phase mixture at a given temperature, the lower a compound's solubility. Therefore, high pressure phase behaviour data may be used to predict the feasibility of separation and obtain an indication of the operating conditions.

Although the phase behaviour of binary mixtures of selected MT and their derivatives in CO_2 have been studied, the phase equilibria data for most of the components of buchu essential oil in CO_2 could not be found in the open literature. Limonene is a ubiquitous terpene, found in essential oils of many different plants, including citrus, from where it derives its name. Data on the phase behaviour of the system limonene + CO_2 can be found in open literature [14–17], and are often used to represent the behaviour of the terpene fraction in essential oils. Pulegone and diosphenol are key OT found in the *A. crenulata* and *A. betulina* essential oils, respectively. Knowledge of their phase behaviour is important in the determination of separability of the terpene fraction from the oxygenated terpene fraction. Terpinen-4-ol is an alcohol-type oxygenated terpene, whose phase behaviour may give an indication of the behaviour of other alcohol type compounds found in buchu essential oil. Therefore, knowledge of the phase behaviour of the four compounds, limonene, pulegone, terpinen-4-ol and diosphenol, may give an indication of the feasibility of deterpenation of buchu essential oil using CO_2, and the process conditions at which the process is likely to be feasible.

The aim of this paper is thus to report, for the first time, phase behaviour data for the systems pulegone + CO_2; diosphenol + CO_2; and terpineol + CO_2. In addition, complementary limonene + CO_2 data are presented in an attempt to resolve the differences between the various literature sources. The first three solutes are oxygenated compounds, while limonene represents the phase behaviour of the MT fraction. The measured data are modelled using Chrastil's equation and this model is used to estimate the SF between different compounds. Based on the experimental data, the possibility of deterpenation of buchu essential oil and the isolation of pulegone using supercritical (SC) CO_2 is considered.

Table 1
GC analysis for essential oil composition of *A. betulina* and *A. crenulata* showing only compounds present at compositions >1% [5].

Component	*A. betulina*	*A. crenulata*
Peak area (%)		
Hydrocarbon monoterpenes (MT)		
Limonene	11.54	11.7
Myrcene	–	1.01
Other (individually <1%)	2.57	1.85
Total MT	14.11	14.56
Oxygenated monoterpenes (OT)		
Cineol	4.43	1.19
Menthone	9.82	2.91
Isomenthone	19.91	3.58
Diosphenol	22.3	–
Pseudo-diosphenol	18.58	–
Pulegone[a]	–	59.3
8-hydroxy-para-menth-4-en-3-one	–	1.03
Hydroxymenthone	–	4.67
Other (individually < 1%)	6.64	2.65
Total OT	81.68	75.33
Sulphur terpinoids		
(Z)-8-mercapto-para-menthan-3-one	2.33	–
(E)-8-acetyl thio-para-menthan-3-one	–	6.83
Total ST	2.33	6.83
Total MT, OT and ST	98.12	96.72
Other compounds	1.88	3.28
Total	100	100

[a] Total of pulegone and isopulegone.