EXTRACTION OF ESSENTIAL OILS FROM VETIVER (*VETIVERIA ZIZANIOIDES*) GRASS

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This thesis (ENCH8FYH) is submitted as a requirement for the degree of Master of Science in Engineering (MScEng) in the School of Chemical Engineering at the University of KwaZulu-Natal.

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As the Candidates Supervisor I agree to the submission of this thesis.

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DECLARATION

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ABSTRACT

Vetiver grass is a viable vegetative absorbent and erosion barrier; in addition the valuable oils extracted from its roots are playing an increasing role in the perfume, food and pharmaceutical industries. The quantity and quality of oil extracted from the vetiver grass depends strongly on location of growth, and the extraction and separation techniques adopted. The aim of this research project is to evaluate whether the harvesting and extracting of essential oils from locally grown vetiver grass would be a feasible business idea, as well as, which extraction technique will give the highest yield of vetiver oil.

The extraction methods tested are solvent extraction, hydro distillation and supercritical carbon dioxide extraction. Due to the lack of supercritical fluid extraction equipment available a large portion of the research project was on the design and setup of a supercritical fluid extraction unit.

The experimental investigations undertaken using solvent extraction in a soxhlet apparatus with hexane as the extracting agent gave an average yield of $\pm$ 1.6% for a 5 hour run which is slightly lower than the yield of 1.91% for a 5 hour run stated in literature. According to the experimental results, yields of up to approximately 2% for hexane extraction can be achieved by increasing the extraction time to 12 hours.

The vetiver roots were also hydro-distilled in a clevenger apparatus for 16 hours (extraction time); this produced a yield of approximately 0.18 to 0.35%. According to literature hydro distillation of vetiver roots in a similar apparatus resulted in an average yield of 1.8% for a 16 hour run. This showed that the heavier components of the vetiver oil were not released during the hydro-distillation extraction.

Research shows that supercritical carbon dioxide extraction (SCE) produces the highest yields ranging from 2.9 to 3.74% when using the recommended parameters of 190 bar and 50°C. Experimentally a yield of approximately 2.3% was achieved by SCE at 180 bar and 40°C. This yield is lower than that seen in literature due to the lower operating temperature and pressure; however SCE gives a higher yield than the other methods tested in this investigation.

The composition of the vetiver oil extracts were analysed using gas-chromatography techniques and this showed that a large percentage of nootkatone is present when using the hydro distillation technique, whilst a large percentage of zizanoic acid was present when using the solvent extraction technique. However a minimal percentage zizanoic acid with higher percentages of nootkatone and khusimol are present in the SCE extracts.

The solvent extraction technique gives high yield with high percentage invaluable zizanoic acid whereas hydro-distillation gives very low yields but no zizanoic acid with high percentages valuable nootkatone and khusimol. SCE gives slightly higher yields of vetiver oil than solvent extraction and it contains very minimal zizanoic acid with higher percentages of nootkatone and khusimol. It was therefore concluded that SCE would be the best extraction method for these particular vetiver roots.

For a pilot scale SCE extractor the total annual sales was estimated as R 453 420 and the total operating costs per annum were estimated to be R 4 839 813. Therefore from this preliminary feasibility study it is seen that the total operating costs far exceed the total annual sales and hence the business is not profitable.

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NOMENCLATURE

Symbols

D	Diameter (m)
H	Height (m)
W	Width (m)
ε	Porosity
ρ_p	Density of the particle ($kg.m^{-3}$)
ρ_b	Density of the bed ($kg.m^{-3}$)

Abbreviations

EWS	eThekwini Water and Sanitation
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS/MS	Gas Chromatography-Tandem Mass Spectrometry
GC – FID	Gas Chromatography – Flame Ionization Detector
HPLC	High pressure liquid chromatography
INR	Institute of Natural Resources
KI	Kovats Index
NCI	Negative chemical ionization mass spectra
NEDFi	North Eastern Development Finance Corporation Ltd.
NRC	National Research Council
PCI	Positive chemical ionization mass spectra
PVC	Polyvinyl chloride
RI	Retention Index
RSM	Response surface method

RT	Retention Time
SCE	Supercritical carbon dioxide extraction
SFE	Supercritical fluid extraction
SWE	Subcritical water extraction
TLC	Thin layer chromatography
VN	Vetiver Network

Subscripts

x = Target Component

n_0 = No. of carbon atoms of standard eluting before x

n_1 = No. of carbon atoms of standard eluting after x

CHAPTER 1: INTRODUCTION

Recently there has been an increase in demand for essential oils extracted from plant material. This can be attributed to the fact that essential oils have multifunctional properties and hence are playing an increasing role in the food, fragrance, agricultural and pharmaceutical industries (Kim et al., 2005).

Vetiver grass also known by the Latin name *Chrysopogon Zizanioides* or its traditional name Khus, is a perennial grass which is part of the Poaceae family (Joy, 2009). The root is the most valuable part of the grass as it forms an intertwined network that stops erosion and it contains the majority of the essential oils which has valuable aromatic and biological properties (Danh, 2007). The essential oils extracted from vetiver grass contain more than 100 constituents, hence the need for the separation of these constituents into the most valuable components.

In developing countries, agricultural grasses are very popular types of crops for rural outreach programmes due to the high value of the essential oils extracted from the grass. Vetiver grass has many uses; it is very easy to grow as it can withstand harsh environmental conditions and does not require large amounts of fertiliser or pruning and therefore it is easily maintained by the user at low cost. It is capable of growing in extreme soil types; this includes sands, shale, gravels, mine tailings and even more toxic soils. It is also very easy to control the spread of vetiver grass as it propagates by root division or slips and is easily removed when no longer required (Islam et al., 2008).

Vetiver grass is easy to establish as a hedge; and it is unlikely to be a host for pests or diseases of any other plants (Njau and Mlay, 2003). It is capable of developing new deep penetrating roots from nodes buried by trapped sediment and continues to grow at the new ground level to form natural terraces. Vetiver grass is native to India (National Research Council, 1993), however it is cultivated to a limited extent in South Africa and is used as a hedge plant particularly in KwaZulu-Natal.

With the growing awareness of the many uses and advantageous characteristics of the vetiver grass it is important to the agricultural and biological engineering fields. The government is starting to appreciate its advantages and this in turn has led many research groups around the country to study its uses and value.

As mentioned above, essential oils (such as vetiver oil) are often used in the food and perfume industry and therefore there is a need to increase the quality of the essential oils extracted. Hence more in depth extraction methods such as microwave assisted extraction; supercritical

carbon dioxide extraction (SCE); and subcritical water extraction (SWE) are now being used (Luque de Castro et al., 1999).

The quality and quantity of vetiver oil varies largely with location of growth and extraction method. Hence the aim of the research project is to evaluate whether locally grown vetiver grass for the extraction of vetiver oil will yield essential oil and if so which extraction technique will give the highest yield.

Possible methods for essential oil extraction and separation are mechanical expression, solvent extraction and distillation as discussed by Danh (2007). Distillation techniques include hydro distillation, steam distillation, and vacuum distillation.

In order to investigate which extraction method will yield the most vetiver oil of the best quality, the following extraction methods were tested; solvent extraction, hydro distillation and supercritical carbon dioxide extraction.

The project scope included the excavation of the vetiver grass from the eThekwini Water and Sanitation departments (Durban Municipality) Newlands Mashu site. As well as the preparation of the vetiver roots for extraction. The solvent extraction and hydro distillation techniques were undertaken in simple laboratory setups known as the Soxhlet and Clevenger apparatus respectively. Due to the lack of supercritical fluid extraction equipment available a large portion of the research project was on the design and setup of a supercritical fluid extraction unit. A brief investigation into the composition of the vetiver oil was also undertaken using GC-MS methods.

CHAPTER 2: LITERATURE REVIEW

2. 1 Vetiver Grass and Vetiver Essential Oil

2.1.1 Background

Table 2. 1. Countries Where Vetiver is Currently Known to Exist (NRC, 1993).

Africa	Asia	Caribbean	America	Pacific	Others
Algeria	Bangladesh	Antigua	Argentina	Fiji	France
Angola	Burma	Barbados	Brazil	Cook Islands	Italy
Burundi	China	Cuba	Columbia	New Caledo	Spain
Comoro	India	Haiti	Costa Rica	Wester	USA
Central Africa Republic	Indonesia	Dominican Republic	French Guiana	Americ an Samoa	USSR
Ethiopia	Nepal	Jamaica	Guatemala	New Guinea	
Gabon	Japan	St. Lucia	Guyana	Tonga	
Ghana	Malaysia	St. Vincent	Honduras		
Kenya	Pakistan	Martinique	Paraguay		
Madagascar	Philippines	Puerto Rico	Suriname		
Malawi	Singapore	Trinidad			
Mauritius	Sir Lanka	Virgin Islands			
Nigeria	Thailand				
Rwanda					
Reunion					
Seychelles					
Somalia					
South Africa					
Tanzania					
Tunisia					
Uganda					
Zaire					
Zambia					
Zimbabwe					

There are two species of vetiver grass found in South Africa, (Grimshaw, 1997) *Vetiveria nigratana* and *Vetiveria zizanioides*. *Vetiveria nigratana* is indigenous to Southern Africa and is found mainly in rivers close to the Okavambo Swamps in Botswana. *Vetiveria zizanioides* was

introduced into South Africa via the province of KwaZulu-Natal in 1860. The latter species is most common and is now widely used in South Africa for soil and water conservation, and land stabilization. *Vetiveria zizanioides* is identical to the Vetiver species found all over the world, namely Australia, USA, Mauritius, South India and Fiji.

Truong and Hart (2001) reported in the Pacific Rim Vetiver Network Technical Bulletin that in 1995 vetiver grass was first recognized for having very absorbent properties and by 1997 this was proven to be fact. Due to the scarce water problems we are facing in the 21st century, more and more vetiver systems are being used for water treatment.

Tony Tantum, a major vetiver grass researcher and promoter in South Africa, built a broad national base of institutional awareness of vetiver (NRC, 1993). In January 1997 the Vetiver Network (VN) agreed to collaborate with the Institute of Natural Resources (INR) in establishing a Southern Africa Regional Vetiver Network. The INR is a non-profit organization affiliated to the University of KwaZulu-Natal which promotes awareness about the advantages and uses of vetiver grass and develops many vetiver programmes (Grimshaw, 1997).

2.1.2 Description of Vetiver Grass

There are two types of vetiver grass (NRC, 1993) that we are aware of today; one originated from North India and one from South India. It is important to distinguish between the two types as the South Indian type is domesticated and is therefore found all over the world, whereas the North Indian type is wild and can become a weed.

Table 2. 2. Comparison between South and North Indian Vetiver Grass (NRC, 1993).

South India	North India
• Domesticated	• Wild
• Non flowering	• Flowering
• Non seeding (or at least non spreading, infertile seeds)	• Sets fertile seeds
• Oil is dextrorotatory (rotates polarized light to the right)	• Oil is levorotatory (rotates polarized light to the left)
• Safe to use for erosion control	• Roots are shallow therefore not suitable for erosion control
• Thicker stem	
• Less branched roots	
• Wider leaves	
• Higher oil content and yield	

Vetiver grass can be described as large clumps of grass that consist of a crown, leaves, stems, roots and sometimes flowers (NRC, 1993). It can grow to a clump diameter of about 300 mm,

an above ground height of 500 to 1 500 mm and a root depth of 3 000 mm. The crown is the part of the grass that connects the stems to the roots. It is buried a few centimetres below the surface of the ground and is dome shaped. The crown is made of debris, plant tissue and rhizomes. It forces the grass leaves and stems to grow in clumps and in an upright position.

Vetiver leaves are long and narrow with a soft section on the top and a hard firm section at the bottom (NRC, 1993). The leaves can be barbed or unbarbed at the top and are therefore often trimmed to leave behind only smooth edges which allows for easy handling.

The stems (culms) of the vetiver grass are strong, hard and lignified; therefore providing strength for the erosion control barrier. The roots are numerous, strong and fibrous. They tend to grow downwards rather than sideways. The mass of roots allows the grass to have a tight hold on the ground which makes it very resistant to adverse weather conditions (NRC, 1993).

If flowers or seed heads do exist they grow to be very large and are purple or brown in colour. The upper section of the flower is male and the lower section is hermaphrodite. Often the seeds are infertile therefore preventing the spread of the grass by seeds (NRC, 1993).

2.1.3 Uses of Vetiver Grass

There are two ways of using vetiver grass (Chomchalow and Chapman, 2003): to make use of the planted vetiver grass or to utilize the harvested vetiver grass. When utilizing the live vetiver grass there are conventional and non-conventional uses, conventional obviously being the most popular uses.

Use of planted vetiver grass:

Conventional uses:

- Soil and water conservation
- Erosion control
- Slope stabilization
- Absorption of heavy metals (utilization of vetiver grass in stabilizing slime dams in the mining industry)
- Wastewater treatment

Non-conventional uses:

- Livestock grazing
- Ornamentals
- Barriers

Chomchalow and Chapman (2003) also state that every few months it is necessary to cut the leaves of the vetiver grass to promote growth and to prevent fire during the dry season. Hence the need to utilize the harvested vetiver leaves and culms to provide an extra income. The vetiver grass can also be grown specifically to harvest the roots of the grass that contain valuable essential oils. The roots, leaves and culms can be used in a processed, semi-processed or non-processed form depending on its application.

Use of harvested vetiver grass:

- Agricultural:
  - Mulch (protective covering placed over soil)
  - Compost (decomposed leaves and culms)
  - Animal feed (young vetiver leaves)
  - Botanical pesticides
- Allelopathy (inhibit growth of other plants)
- Insect Repellent:

  Nootkatone, α-vetivone, β-vetivone, khusimone, zanal and epizizanal are components known to exist in vetiver oil (Refer to section 2.1.4) which have insect repelling abilities and are non-toxic to humans due to their natural origin (Henderson et al. 2003).
- Handicrafts (known to have cooling properties)
- Construction (e.g. thatched roofs)
- Medicinal (traditional)
  - Antifungal, anti-inflammatory and antioxidant (β and α - vetivones) (Danh, 2007)
- Fragrance:
  - Perfumes – Fixative or as a fragrance itself

    e.g. Guerlain‟s „Vetiver‟, Chanel‟s „Coco‟, Dior‟s „Miss Dior‟, Yves St. Laurent‟s „Opium‟ and Givenchy‟s „Ysatis‟ (Dowthwaite and Rajani, 2000)
  - Aromatherapy- Vetiver oil is known to have several beauty, health and emotional benefits
  - Potpourri
- Flavour and preservatives in the food industry (Lavania, 2003)
  - Ice cream
  - Beverages
  - Food preservative
  - Spices

- Energy Source (Ethanol)
- Raw material for pulp and paper industry

Vetiver Used for Wastewater Treatment

After primary treatment of wastewater there are still significant amounts of contaminants and nutrients in the water and therefore further treatment is required to reduce these contaminants and nutrients to an acceptable level. According to Peavy et al. (1985) constructed wetland can be used to remove these contaminants from wastewater. The performances of the wetlands are therefore improved by using vetiver grass. The vetiver grass roots provide a large surface area for colonization of wastewater by heterotrophic bacteria that degrade organics materials and at the same time the vetiver roots create a hostile environment for other pathogenic organisms in the wastewater (Chomchalow, 2001).

Vetiver grass can serve as a sink for wastewater as it can be grown in pontoons on wastewater ponds; it can be grown in constructed wetlands; or used for irrigation of the vetiver crops. Either way a resource is produced from a waste product.

When vetiver grass used in wastewater treatment is harvested and used for the extraction of vetiver oil, the extraction and purification processes ensure that the products are free from pathogens so the wastewater does not need to be disinfected prior to contact with the vetiver grass.

Truong and Hart (2001) discuss the suitability of using a vetiver grass system for wastewater treatment. They found that due to the following morphological and physiological features of the vetiver grass, that it is indeed highly suitable.

Morphological features:

- Stiff and erect stems which can withstand high velocity flows; therefore when planted close together can form a living porous barrier.
- Its deep root system allows the plant to grip tightly into the ground and to withstand adverse weather conditions
- It has many fine root branches which allows for a large surface area for absorption of contaminants and nutrients

Physiological features:

- High tolerance to heavy metals
- High tolerance to adverse weather conditions

- High tolerance to adverse soil conditions
- High absorption rate of nitrogen and phosphorous
- Highly tolerant to pesticides
- Regenerates rapidly
- High water use rate

According to Truong and Hart (2001) vegetative methods are the only feasible and practical methods available for large scale reduction or disposal of wastewater. Recently it has been found that using vetiver grass as a vegetative absorbent is highly effective; this is due to its ability to absorb high amounts of nitrogen, phosphorous and organic compounds, which are key elements in water pollution (refer to Figure 2.1). Vetiver wastewater treatment systems can be used for industrial or domestic effluents as well as landfill leachate.

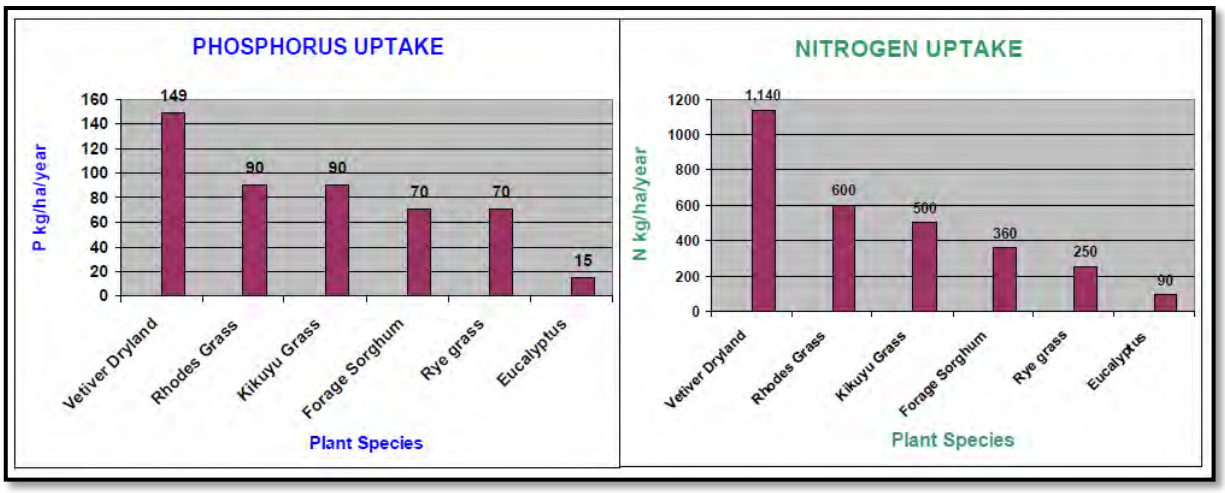


Figure 2. 1. Phosphorous and Nitrogen uptake rates (Truong and Hart, 2001).

There are two common methods (Truong and Hart, 2001) for this treatment: land irrigation systems or wetlands. In land irrigation the wastewater is used directly to irrigate the vetiver grass. This promotes growth of the vetiver which can then be harvested and used to generate an income while at the same time serving as a sink for the wastewater. The wastewater could also be used to irrigate other types of plants and lawns which serve as a sink for the wastewater. However one needs to prevent any contaminants in the wastewater from seeping into the river systems; therefore vetiver barriers are preferred.

The wastewater can also be collected in a natural or constructed wetland where the vetiver system is grown on pontoons on the surface of the wetland or on the surrounding banks. Again the vetiver grown can be harvested and used for other uses.

According to Headley and Tanner (2006) "*Constructed treatment wetlands are engineered systems designed to enhance the process and interactions that occur in natural wetlands between water, plants, microorganisms, soils and the atmosphere in order to remove contaminants from polluted waters in a relatively passive and natural manner*."

2.1.4 Vetiver Essential Oil

Essential oils are generally a mixture of organic compounds that are located throughout different plant material. This oil is essential to the plant hence the name essential oil and is found in many parts of the plant such as the sacs, cells, glands or even ducts inside the roots, stem, bark, flowers, seeds or leaves (Dowthwaite and Rajani, 2000).

Vetiver essential oil is amber to dark brown in colour; it is one of the most viscous of the essential oils and therefore has a low evaporation rate and it is also soluble in alcohol (Lavania, 2003). This allows the vetiver essential oil to be a perfect candidate for use in the perfume industry, especially as a fixative. Vetiver oil is known to blend well with patchouli, sandalwood, jasmine and many other essential oils; however it is often diluted to prevent the odour from dominating the blend (Bhatwadekar et al., 1982).

The odour in most essential oils vary widely with the natural elements and hence location (Dowthwaite and Rajani, 2000). Vetiver essential oil is known for its earthy woody balsamic odour; however the odour can also be more sweet and roseate (Lavania, 2006). The vetiver root oil varies with the natural elements in which it grows because it is composed of sesquiterpenes and sesquiterpene derivatives which have a high chance of polymerisation and hence polymerise to different degrees depending on the natural elements that it is exposed to (Chowdhury et al., 2002).

Like most essential oils the composition of the vetiver essential oil is extremely complex, it is known to contain more than 100 sesquiterpene compounds and their derivatives (Lavania, 2006). Vetiver oil is rich in C_{15} sesqui-terpeniods which can boil at over 200°C (Dowthwaite and Rajani, 2000).

The main constituent of the vetiver essential oil includes (Lavania, 2003):

- Sesquiterpene hydrocarbons, e.g., cadenene, clovene, amorphine, aromadendrine, junipene
- Sesquiterpene alcohol derivatives, e.g., vetiverols – khusimol, epiglobulol, spathulenol, khusinol
- Sesquiterpene carbonyl derivatives, e.g., vetivones – vetivone, khusimone
- Sesquiterpene ester derivatives, e.g., khusinol acetate

Sesquiterpenes are a class of terpenes (organic compounds found in plants) that consist of three isoprene (C_5H_8) units and have the molecular form $C_{15}H_{24}$ (National Library of Medicine, 2011). Sesquiterpenes have high molecular weights with low vapour pressure (Danh. 2007). The most valuable components found in the vetiver oil have the highest boiling points and therefore are not easily vaporised for collection (Chomchalow, 2001).

The three main odour influencing constituents are known to be α-vetivone, β-vetivone and khusinol (Bhatwadehar et al., 1982).

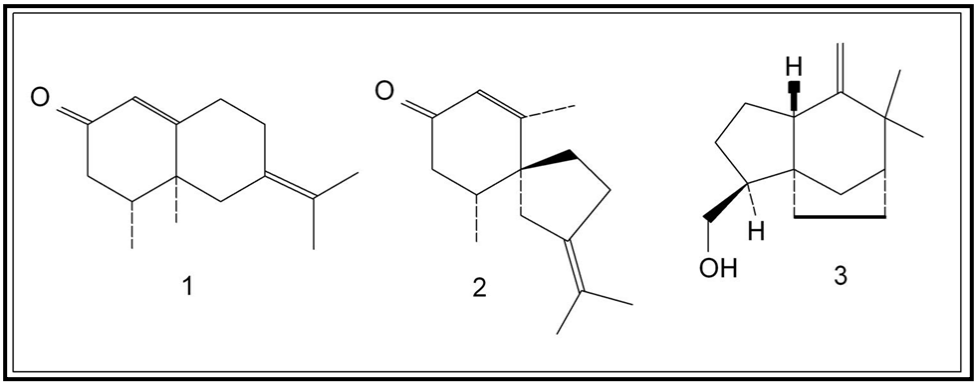


Figure 2. 2. 1: α-Vetivone, 2: β-Vetivone and 3: Khusinol molecular structure (Ohloff, 1994).

The top 5 major components identified in vetiver oil according to gas chromatography area % given in literature are as follows:

1. Zizanoic acid 15-32 %
2. Khusimol 7-15 %
3. Isovalencenol 5-9%
4. α - Vetivone 4-8%
5. β - Vetivone 0-3%

The above conclusion was taken from papers by Danh et al. 2010 and Martinez et al. 2004, both of whom used vetiver grass grown in Brazil for their extractions.

2.1.5 Market Interest in Vetiver Essential Oil

According to TradeInvest South Africa (2008) 65% of the world‟s production of essential oils is produced by developing countries such as India, China, Brazil, Indonesia, Mexico, Egypt and Morocco. In recent years there has emerged an essential oil industry within South Africa.

The essential oils industry is very popular for developing countries as it is suitable for large scale corporate production or as a small scale domestic production. The extraction of essential oils from crops is used for rural upliftment programs as the process of extracting the oils is very laborious. In addition essential oil production is advantageous as it has a high turnover and profit margin, they do not perish, the final production is low in volume and hence can be transported easily and the distribution chain is characterised by long term relationships between buyers and suppliers (Cacadu, Trade and Investment, 2009).

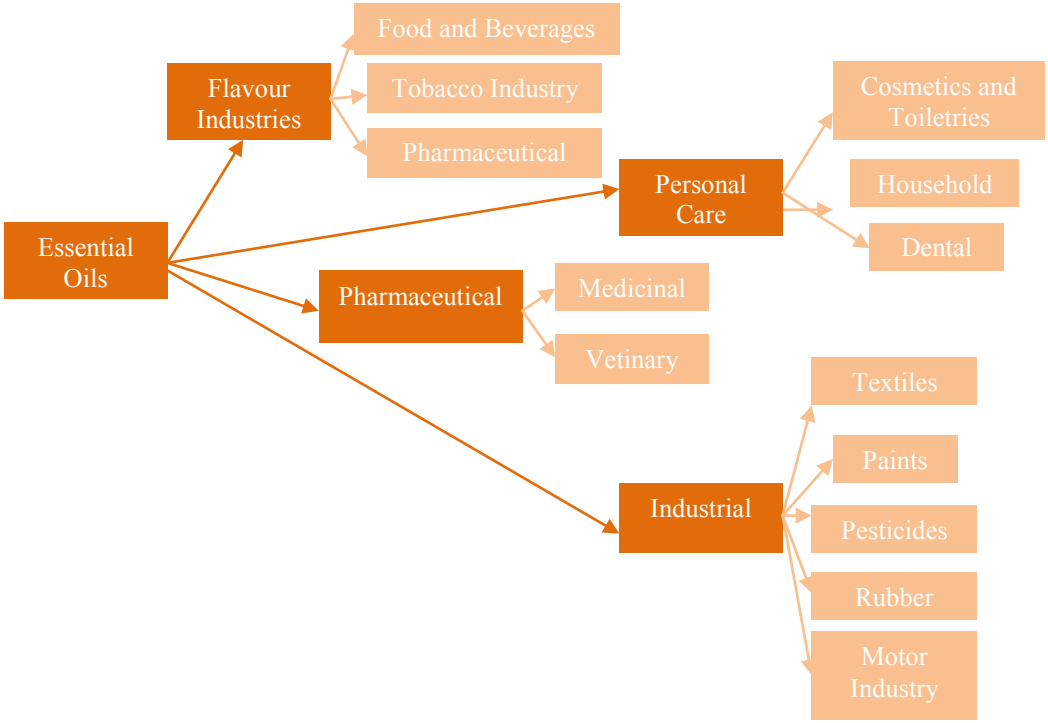


Figure 2. 3. Essential Oils Value Chain for Buyers (Cacadu, Trade and Investment, 2009).

When looking at the essential oils value chain for buyers (refer to Figure 2.3) one can see that the uses stated in section 2.1.3 fit into all four of the main sectors that essential oils are used in and hence one can acknowledge the value of vetiver essential oil. Haiti, Indonesia and Réunion produce most of the world‟s vetiver oil, with China, Brazil, India and some other nations producing smaller quantities (NRC, 1993; Lavania, 2003). The annual production of vetiver oil worldwide is estimated to be 250 tons.

There are many suppliers of vetiver oil within South Africa, however the majority of suppliers are importing the oils from the above mentioned countries (refer to Table 2.3).

Table 2. 3. Annual Demand of vetiver essential oil/ tpa (NRC, 1993).

United States	100
India	100* (of which 80% is met by import)
France	50
Switzerland	30
United Kingdom	20-25
Japan	10
Germany	6
Netherlands	5
Other	30-40

*Ref.: NEDFi (2005)

The statistics reported in Table 2.3 by the NRC in 1993 are outdated; however it shows a gap in the market for vetiver essential oil. According to the NRC (1993) these figures should have stayed roughly the same over the years due to the fact that vetiver oil is expensive and therefore cedar wood oil was being used as a substitute. However, according to the NEDFi (2005) the above prediction was proved incorrect and the world market demand for vetiver essential oil is increasing day by day. This is due to the fact that vetiver has a unique odour, it cannot be substituted with reconstituted oil and it cannot be made synthetically. Vetiver essential oil is found in large proportions in 20% of all men‟s perfumes and 36% of western perfumes (Danh, 2007). Meschede (2009) states that, “Although vetiver essential oil has a good demand, it is still facing a tight supply”. In the publication by Dowthwaite and Rajani (2000) these researchers label vetiver oil as being „high priced oil‟.

An investigation into the typical selling prices of vetiver oil from various suppliers was undertaken and from this it was reported that the selling price varies between R 2 000 and R 20 000 per kg of vetiver oil (refer to Appendix A2 / Currency Conversion: 1 US Dollar ($) = 7 South African Rand (R) [Average of latest trends]). The large variation in the selling price is due to the large variations in the quality of vetiver oil produced from different regions and suppliers.

The NEDFi (2005) reported a study on the economics of a vetiver cultivation grown in India (refer to Appendix A3). The study was done on a per hectare per 18 month duration basis and showed the net returns achievable. A net return of R 10 825 (1 Indian Rupee (Rs) = 0.15464 South African Rand (R) [15/02/11]: Original source in Rs) was reported for a plant that produces vetiver oil from the vetiver cultivation and a plant that produces only dry vetiver roots can achieve a net return of R 5 613 (1 Indian Rupee (Rs) = 0.15464 South African Rand [15/02/11]: Original source in Rs). The calculations above were based on a dry root mass of

3 000 kg vetiver roots per hectare and a vetiver oil recovery of only 0.4% on a dry basis. It is assumed that the vetiver oil recovery can be improved by maximizing the extraction technique and hence increasing the net return of vetiver oil. The expenditures used in the study are dependent on the cost of raw materials and labour within each country.

2.1.6 Growing of the Vetiver Grass

Vetiver grass is propagated mainly by root division or slips (NRC, 1993). These slips or tillers are cut off the main clump of the vetiver grass and planted as seedlings in the ground (50 – 80 mm deep, NEDFi, 2005). These slips are planted close together typically between 10 – 30 cm apart depending on whether hedge formation is an aim or not. The freshly planted slips should be irrigated if not planted during the rainy season.

Figure 2. 4. Bare Root Slips and Tube Stock (Truong et al., 2008)

Vetiver grass will grow in any soil type (refer to Table 2.4) however for fast growth a rich, well-drained soil with loose texture is recommended (NEDFi, 2005). The same applies for the climate; even though vetiver grass can withstand adverse weather conditions (refer to Table 2.4), it has an optimum growing climate. Vetiver grass grows best under warm and damp conditions (by the edge of water) and therefore prefers a warm summer climate with well distributed rainfall (NEDFi, 2005). Vetiver does not grow well in the shade; it needs sunlight to thrive especially when first planted.

The microbes and bacteria present in the soil surrounding the vetiver roots react with the vetiver oil within the roots and hence sesquiterpenes undergo bio-conversion into oxygenated sesquiterpenes (NEDFi, 2005). The oxygenated sesquiterpenes give the vetiver essential oil its unique odour. Therefore the odour in the vetiver oil produced is related to the soil conditions for growth and hence the location of growth. Synthetic fertilizers and pesticides kill the microbes present in the soil and hence the process of bio-conversion is stopped which in turn yields less valuable vetiver oil. It is therefore recommended to only lay fertilizer every 18 months (NEDFi, 2005) (approximately once at the beginning of plantation).

Table 2. 4. General Tolerance Range for Growing Vetiver Grass (Truong and Hart, 2001)

Adverse Soil Condition:	
Acidity	pH 4.2
Alkalinity	pH 10.5
Aluminium Level (Al Sat. %)	80-87
Heavy Metals/ $mg.kg^{-1}$:	
Cadmium	22
Copper	174
Lead	3123
Zinc	3418
Altitude/m	2800
Climate:	
Annual Rainfall/mm	250-5000
Frost (Ground Temperature) /^{o}C	-22
(Soil Temperature)/^{o}C	-10
Heat Wave/^{o}C	60
Fertilizer	Nitrogen and Phosphorous, Farm Manure
Palatability	Cows, cattle, goats, sheep, pigs and carp
Nutritional Value/%	Crude Protein 3.3
	Crude Fat 0.4
	Crude Fibre 7.1

For maximum growth it is advisable to trim the aerial portion of the grass at the start of winter; this encourages tiller and root growth (NEDFi, 2005). Once the vetiver grass is between 15 - 18 months old it is ready for harvesting. Between 15 and 18 months the roots of the grass are thicker and mature, therefore yielding more oils of a higher quality. If left longer than two years the roots become woody, hence loosing essential oil content. If harvested before 15 months the roots are too immature for extraction and they yield vetiver oil of poor quality with an earthy odour that can fade with time (NEDFi, 2005).

Harvesting should be done during the dry season preferably at the beginning as the ground may become too dry and hard towards the end of the dry season making it difficult to dig up the roots (NEDFi, 2005 and Lavania, 2003). After harvesting, the roots can either be extracted while they are still fresh or they can be left to mature for 12-24 months to increase the vetiver oil yield by enzymatic processes (Dowthwaite and Rajani, 2000).

One can tell whether the roots are ready for extraction when they are thick, hard, long, wiry, and give a bitter taste when chewed. The stem is first cut at a height of 15 – 20 cm and then the root is dug out of the ground with a spade or a tractor (NEDFi, 2005).

As discussed above in section 2.1.3 vetiver grass can be grown on pontoons which serve as the floating mat that allows the vetiver grass to grow on the surface of the constructed wetlands or grown on the wetland banks.

The vetiver grass slips are planted in some sort of matrix or soil media on the pontoon. The roots again grow downwards into the water where they acquire nutrients directly from the water to keep the plant alive, while at the same time removing unwanted nutrients and contaminants from the wastewater. This pontoon set up is known as floating treatment wetlands (Headley and Tanner, 2006). The vetiver roots grown on pontoons are shorter in length due to the fact that nutrients are readily available to the root and hence root spread downward is unnecessary (refer to Figure 2.5).

When designing a floating pontoon system one must take into account durability, functionality, environmental sensitivity, weight, buoyancy, anchoring, flexibility and cost (Headley and Tanner, 2006). Generally a square, triangular or rectangular framed pontoon is used and the number of pontoons used depends on the size of the lake, pond or wastewater tank. Materials used include polyvinyl-chloride (PVC) pipes, polystyrene sheets or foam, bamboo or inflatable vinyl pillows.

Figure 2. 5. Roots of the vetiver grass grow in water (left) and in soil (right) (Truong et al., 2008)

2. 2 Extraction of Vetiver Essential oil

According to Luque de Castro et al. (1999) the techniques for extraction of essential oils from plant matter can be classified into three techniques: continuous conventional, discontinuous conventional and non-conventional. The type of extraction method used directly affects the quality, yield and odour of the essential oil.

The steps below describe the mass transfer mechanism for the extraction of essential oils from plant material (Talansier et al., 2008):

i. Constant extraction rate – The external surface of the particles (plant material) are completely covered with oil.

ii. Falling extraction rate – Phase where the external surface oil has been depleted by mass transfer into the extracting fluid and the surface area of the particles are only partially covered.
iii. Diffusion period – No oil is present on the external surface of the particle and hence diffusion occurs.

According to Talansier et al. (2008) 70% of extracted oils are extracted in the constant and falling rate extraction periods of which 50% is extracted in the constant rate period. Therefore the process can be modelled considering convective processes only (i.e. neglecting the diffusion process). However, Chomchalow (2001) states that vetiver roots do not easily yield oils as the oils are located in the inside root tissue and hence the slow physical process of diffusion must occur before oils are extracted. This and the fact that the vetiver oil consists of high molecular weight Sesquiterpene (refer to Section 2.1.4) contributes to the long extraction times necessary for extraction of the vetiver oil from vetiver roots.

2.2.1 Distillation (Continuous-Conventional)

Distillation is the process in which the raw material (prepared vetiver roots) is heated in order to separate the volatile and non-volatile components by collecting both the top product (distillate) and the bottom product (bottoms), which is condensed and recycled respectively. The type of distillation is defined by the heating medium used. There are four types of mediums employed when distilling the essential oils from the vetiver grass (Douglas et al., 2005). These include:

Hydro Distillation

Also known as water distillation, it is the simplest and most common method of distillation. The raw material is mixed with water in a still pot and heated at the bottom which causes the water to vaporise and take with it the valuable oil extracts (refer to Figure 2.6). The vaporised water and extracts are then condensed into an oil separator where the extracts can be separated. A perforated grid is used to prevent the raw material from settling to the bottom of the pot and becoming overheated. The raw material must also be agitated at all times to promote extraction. This method is not preferred due to the heat and water damage imposed on the extracts.

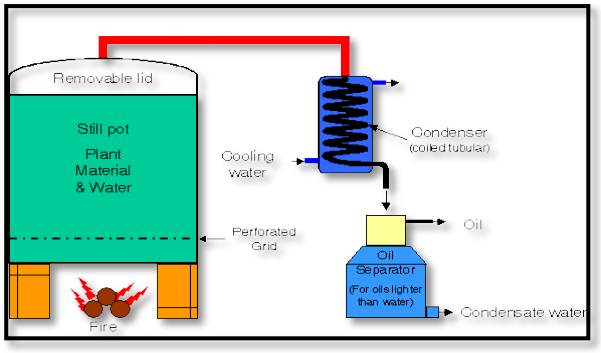


Figure 2.6. Schematic of a Hydro Distillation Setup (Douglas et al., 2005).

Hydro distillation on a laboratory scale, for the extraction of essential oils from plant material is commonly performed in a Clevenger apparatus (refer to Figure 2.7). This apparatus is better suited for separation of the extracts from the water due to the fact that the small amount of extract can be collected easily in the collecting arm.

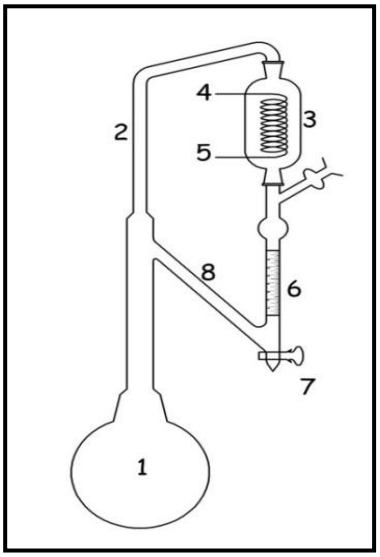


Figure 2.7. Diagram of a Clevenger Apparatus.

1. Round bottom heating flask, 2. Distillation path, 3. Condenser, 4/5. Cooling water in/out, 6. Oil water separator, 7. Tap, 8. Recycle arm.

The water and plant material is placed in the bulb flask (1) and is brought to a boil. The vapours then flow (2) into the condenser (3) where they are condensed and collected in an oil/water separating arm (6). The condensed water is then allowed to flow (8) back into the bulb flask (1) for recycle and the extract which is less dense than water is collected in the arm (6). After the desired extraction time the water is drained off the bottom of the column using the tap (7) and the extract is collected.

Water and Steam Distillation

The raw material is packed into the still pot on a perforated plate and water is boiled beneath the plate (refer to Figure 2.8). Therefore the water vapours pass though the raw material while extracting the valuable extracts. The vapours are also condensed and passed to an oil separator. This method of distillation produces a higher yield with a better quality extract due to the fact that there is no water damage however thermal degradation can still occur.

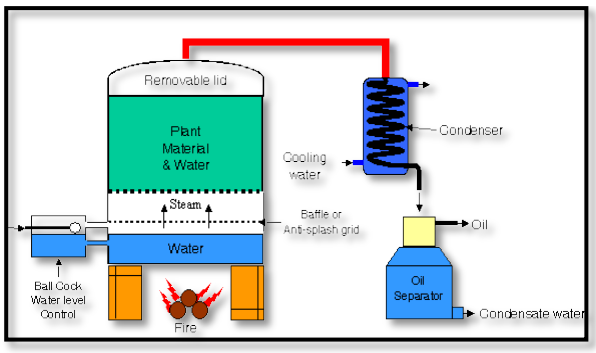


Figure 2.8. Schematic of a Water and Steam Distillation Setup. (Douglas et al., 2005).

Steam Distillation

In this technique the steam is provided by an external boiler source. The raw material is packed onto a perforated plate and the steam is passed over it in order to collect the valuable extracts which are then condensed and sent to the oil separator (refer to Figure 2.9). Although the capital and operating cost will be more for this method, by supplying an external steam source one can control the amount of steam and the temperature of the steam passing over the raw materials and therefore thermal degradation can be controlled.

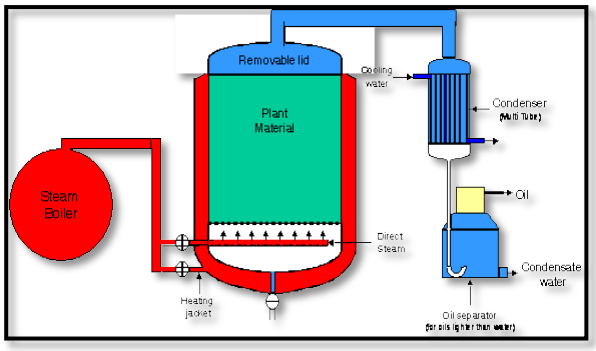


Figure 2.9. Schematic of a Steam Distillation Setup (Douglas et al., 2005).

Vacuum Distillation

In vacuum distillation the pressure above the raw material is reduced to less than the vapour pressure of the raw material causing the least volatile components to evaporate at lower temperatures.

According to Douglas et al. (2005) distillation is the most economical extraction method for essential oils from plant material. This is due to the simplicity of the process, it is affordable and can be done close to where the plant is being harvested. However this method has many disadvantages such as incomplete extraction of oils from the plant material; requirement of a post-extraction separation technique from water, as well as high operating temperatures which cause losses of thermo labile components and promotion of hydration reactions of chemical constituents (Danh et al., 2009 and Danh, 2007). According to Luque de Castro et al. (1999) further disadvantages include low selectivity and long extraction times.

Steam distillation is still the most commercially accepted method of extraction of vetiver oil. Although steam distillation is expensive, it operates at high temperatures and pressures which are needed to rupture the cells of the vetiver roots and hence remove the heavier components of the vetiver oil. In order to remove the vetiver oil compounds (Sesquiterpenes, >200°C boiling point) it is recommended to use 3 bar steam pressure for 18-24 h (Dowthwaite and Rajani, 2000).

2.2.2 Solvent Extraction (Continuous or Discontinuous-Conventional)

Common solvent extraction uses a pure organic or mixed organics to extract the valuable extracts from the plant material. Typical solvents include ethyl acetate, diethyl ether, methanol, ethanol and hexane. This procedure is normally done in a Soxhlet extractor (Danh et al., 2009) in which the solvent is continuously refluxed though the raw material to collect the extracts (refer to Figure 2.10).

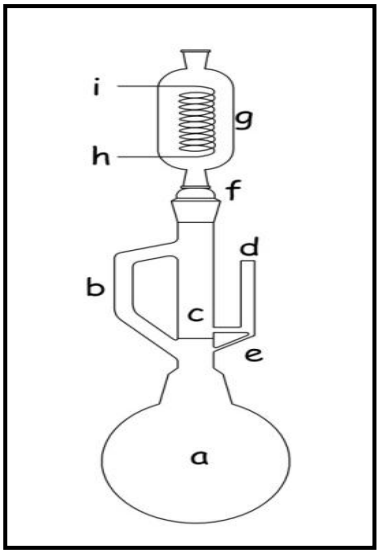


Figure 2.10. Diagram of a Soxhlet apparatus.
Heating/collecting flask, b. Distillation path, c. Thimble (bed for the plant material), d. Siphon top, e. Siphon exit, f. Expansion adapter, g. Condenser, h/i. Cooling water in/out

The solvent is heated in the round bottom flask (a) at the bottom of the apparatus until it begins to vapourize. The vapour flows up the thin outer chamber on the side (b) and enters the soxhlet chamber which is packed with plant material (c). The vapours then flow up further into the condenser (g) which is placed onto the top of the soxhlet chamber and are condensed. The warm solvent then flows though the packed bed while extracting valuable extracts. Once the bed is full of solvent, the solvent with extract is allowed to flow out the siphoning tube (d/e) and finally down back into the bulb for recycle.

The extracts are removed from the flask and left to evaporate until no solvent is present and the essential oil can be analysed. According to Luque de Castro et al. (1999) the disadvantages of solvent extraction are the long extraction times, low selectivity, unfeasibility for automation and the presence of toxic residues in the extract. Therefore solvent extraction is often undertaken on fragile plant material that could be destroyed by the high temperatures used during distillation.

Hexane is the common choice of solvent as it has a low miscibility in water and after evaporation it leaves insignificant amounts of residues.

2.2.3 Mechanical Expression (Discontinuous-Conventional)

Prior to the discovery of distillation most essential oils were expressed mechanically or cold pressed (Sellar, 2001). It is the simple process of heating the plant material to low temperatures and then physically pressing the essential oil out. Today mechanical expression is used mainly for citrus peels and is unpopular due to the low extraction yield.

2.2.4 Microwave Assisted Techniques (Non-Conventional Technique)

The microwave assisted extraction technique is essentially solvent extraction which utilizes microwave energy to heat the solvent and raw material, thereby increasing the mass transfer rate of solute into the solvent. According to Mandal et al. (2007) a microwave is used to heat the microscopic traces of moisture found inside the plant cell therefore causing it to swell and burst allowing valuable extract to mix with the solvent. The process can be enhanced further by impregnating the raw material with the solvent to increase its liquid content.

High temperature can be reached by microwave radiation which can degrade the thermolabile components in the extracts. Hence microwave transparent solvents such as hexane and chloroform can be used to reduce thermolabile degradation (Mandal et al., 2007). The advantage of microwave assisted techniques is that they use considerable less solvent for the extraction therefore reducing the amount of evaporation needed to concentrate the extract and amount of residues left in the extract.

2.2.5 Supercritical Carbon Dioxide Extraction (SCE) (Non-Conventional Technique)

Supercritical carbon dioxide extraction is solvent extraction using a supercritical fluid as a solvent. According to Luque de Castro et al. (1999) supercritical fluid extraction (SFE) is based on the enhanced solvating power of fluids above their critical points. Supercritical fluid extraction is becoming increasingly more popular due to the fact that the world is becoming more and more environmentally orientated. SFE is a less energy intensive process and it produces a cleaner product that has a higher quality which adheres to the stricter regulations now in place.

Carbon dioxide is a popular fluid to be used (McHugh and Krukonis, 1986) because it is nontoxic, non-flammable, inert, readily available, inexpensive, easily removed after extraction and has preferred critical properties (low pressure and ambient temperature; refer to Figure F.1, in appendix F).

The grade of CO_2 used during extraction should be selected with the application of the extracts in mind. An industrial grade CO_2 would not be suitable for the food and fragrance industries as there is potential risk of contaminants within the CO_2 and hence a food/medical grade CO_2 should be used, which abide by the hygiene standard laws which are enforced when working in the food and fragrance industries (which includes glass lined holding vessels and proper cleaning procedures).

According to Danh et al. (2007 and 2009) supercritical carbon dioxide extraction is advantageous over conventional techniques as it operates at a lower temperature, therefore reducing thermal degradation and it eliminates the problem of residual solvents in the extracts. The SCE method also allows the extraction to retain the organoleptic characteristics of the starting plant material. It can be said that SCE is a clean technology and therefore is very popular for extraction of oils for the use in the food industry (Martinez et al., 2004).

Herrero et al. (2006) states that at a supercritical state the liquid and the gas phases are indistinguishable hence the fluid takes on the density of the liquid phase and the viscosity of the gas phase. Supercritical fluids therefore have low viscosity and high diffusivity (refer to Figure F. 4 and Figure F. 3 respectively) making it easier to diffuse through solid materials which in turn give faster and better extraction yields.

The SCE process takes place in an extracting column as shown in Figure 2.11. The carbon dioxide is pumped at the required conditions into the line where it is heated and then sent to the stainless steel column packed with the vetiver grass. The pressure at the outlet valve is decreased causing the extract to be collected in the flask which is cooled by an organic solvent (Martinez et al., 2004).

The extraction period can be divided into two stages, the dynamic and the static stage. The static stage is when no flow is exiting the system and hence pressure is building up within the system. The dynamic stage is when the outlet valve is opened slightly to allow for a continuous flow while still maintaining a constant pressure inside the system. The efficiency of the extraction depends on time, pressure, temperature and particle size therefore making the system multivariable and hence difficult to optimize (Danh, 2007).

Supercritical carbon dioxide is a hydrophobic solvent and therefore to remove the more hydrophilic components of the essential oil such as the oxygenated terpenes and sesquiterpenes, a polar co-solvent such as ethanol can be used (Talansier et al., 2008). This will therefore increase the yield of the extract.

When deciding on the operating parameters for SFE one must consider the critical point of the solvent being used. It is necessary to keep the temperature as low as possible to avoid thermal degradation and to keep the density of the solvent up therefore increasing solvating power. However by increasing the temperature at a fixed pressure, it causes the vapour pressure of the extracts to increase therefore making them diffuse into the fluid phase easier (Reverchon and De Marco, 2006).

The operating pressure is also a tricky decision as the higher the pressure the better the extraction but the lower the selectivity. Other operating parameters include particle size and CO_2 flowrate. Both these parameters influence the mass transfer and hence the extraction (Reverchon and De Marco, 2006).

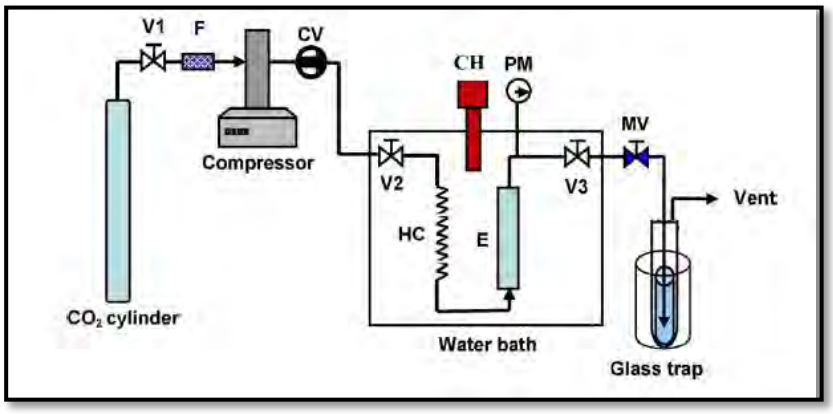


Figure 2.11. Schematic diagram of a Supercritical extraction setup. V1, V2, V3: stopping valve; F: filter; CV: check valve; HC: heating coil; E: extraction vessel; CH: circulating heater; PM: pressure meter; MV: micro-metering valve (Danh et al., 2009).

The engineering design of a supercritical extraction setup requires the knowledge of the thermodynamics and kinetic constraints that apply to the system. According to Ferreira et al. (2002) the mass transfer mechanism for SFE extraction is not fully understood due to the complex flow patterns within the bed and even more so when extracting essential oils as the essential oils consist of many components. Hence the interactions between the solvent and the solute are difficult to predict.

Various publications such as Sovova (2005), Ferreria and Meireles (2002) and Bhupesh et al. (1996) focus on the mathematical models and extraction curve evaluation for the extraction of natural product. Most of which split the process into two extraction periods the first one governed by phase equilibrium and the other governed by internal diffusion. The concept is further broken down into intact and broken cells. The model is versatile for various natural products however may be more complex for some products (Sovova, 2005).

2.2.6 Continuous Subcritical Water Extraction (SWE) (Non-Conventional Technique)

Sub critical water extraction uses water in its subcritical state as a solvent. According to Herrero et al. (2006) SWE is operated with water in its subcritical liquid state at high temperatures (100 to 374°C) and high pressures (10 to 60 bar). The extraction principle used in the SWE technique is based on the variability of the dielectric constant with temperature. The dielectric constant is decreased by increasing temperature; however with water as a solvent when increasing temperature vaporisation will occur hence the need to operate the system at high pressures. At low dielectric constants water becomes a more efficient solvent hence allowing a better and faster extraction. This method of extraction is also advantageous as it is environmentally friendly.

The SWE process takes place in an extracting column as shown in Figure 2.12.

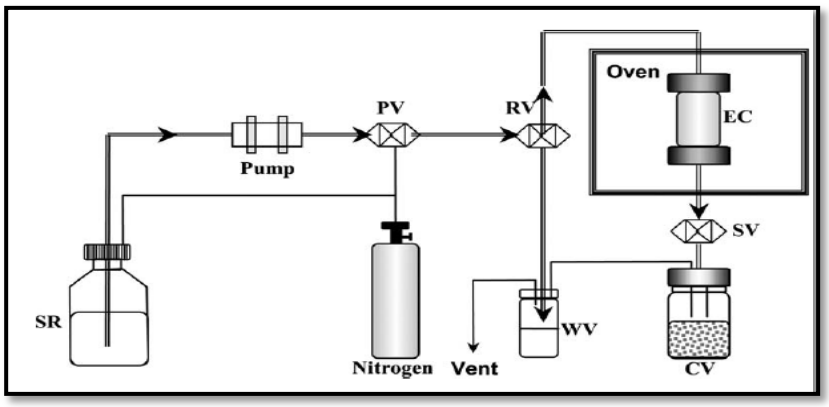


Figure 2.12. Diagram of a subcritical water extraction system.
SR, solvent reservoir; PV, purge valve; RV, pressure relief valve; EC, extraction cell; SV, static valve; CV, collector vial; WV, waste vial (Herrero et al., 2006).

The extraction set up for SCE and SWE as seen in Figure 2.11 and Figure 2.12 respectively allows for control of pressure and flow of the solvent which therefore increases the selectivity of the extraction, hence providing further advantages over conventional techniques where no control is possible.

2.2.7 Comparison of the Extraction Techniques

Table 2.5. Optimum operating parameters and yields for the extraction of vetiver oil using various techniques found in literature.

	Danh et al. 2009	Danh et al. 2010	Talansier et al. 2008	Martinez et al. 2004
Hydro-Distillation:				
Apparatus	Clevenger	Clevenger	Schilcher	Clevenger
Weight of roots/ g	20	30	-	50
Extraction time/ h	12	12	3	16
No. Extractions	3	3	-	-
Operating Temp/ °C	100	100	100	100
Operating pressure	Ambient	Ambient	Ambient	Ambient
****Yield /%**	0.31*	1.69	1.4	1.8
Solvent Extraction:				
Apparatus	Soxhlet	Soxhlet		
Weight of roots/ g	20	30		
Solvent	Hexane	Ethanol	-	-
Vol. of Solvent /ml	300	500		
Extraction Time/ h	5	5		
****Yield /%**	1.91	15***		
Supercritical Fluid Extraction:				
Size of Column (SS) /ml	50	50	5	100
Weight of roots /g	10	10	Packed full	30
Flowrate of CO_2	2 ml/min	2 ml/min	0.09 - 0.12 g/s	0.069 g/s
CO_2 pump temp /°C	4	4	-	-
Static time / min	30	15	5	3
Dynamic time /min	100	105	300	60
Operating Temp/°C	50	50	40	40
Operating pressure /bar	190	190	200	200
****Yield /%**	1.38	3.74	2.9	3.2
Supercritical Fluid Extraction with an ethanol co solvent:				
Size of Column(SS) /ml		50	2.35	
Weight of roots /g		10	20	
Flowrate of CO_2		2 ml/min	0.09 - 0.12 g/s	
CO_2 pump temp /°C		4	-	
Static time / min	-	15	5	-
Dynamic time /min		105	300	
Ethanol Vol. %		15	10	
Operating Temp/°C		50	40	
Operating pressure /bar		190	200	
****Yield /%**		5.9	4.7	

* In 2009 Danh et al. used roots that where only 7 months old whereas in 2010 Danh et al. used roots that where older than 4 years.

** Yield represents weight of collected oil over dry weight of roots.

*** Large amounts of heavy waxy components in extract.

Martinez et al. (2004) studied the valorisation of Brazilian vetiver oil. They studied the extraction method, the chemical composition of the extracts, their sensorial characteristics and the possibility of chemical transformations of the product. The extraction methods tested were hydro distillation and supercritical carbon dioxide extraction (SCE).

Hydro distillation was undertaken using operating parameters as stated in Table 2.5. There were five runs for hydro distillation, initially with the untreated vetiver roots and then with the four different treated roots. It was expected that pre-treatment of the vetiver roots would improve the contact between the oil and extraction medium and hence increase yield. Possible pre-treatment methods include, milling in liquid nitrogen, treatment with sodium hydroxide, enzymatic treatment (Celluclast and Pectinex Ultra SP-L) and combined sodium hydroxide and enzyme treatment.

However the untreated roots, the roots treated with sodium hydroxide and the roots milled with nitrogen all gave an oil extraction yield of approximately 1.8%. The roots treated with enzymes gave a yield of approximately 1.9% and the roots treated with sodium hydroxide and enzymes gave a yield of approximately 1.7% (refer to Table B.1). This proves that pre-treating the roots does not improve oil yield significantly. Hence it is not worth the cost and time that it would take to pre-treat the roots; therefore pre-treatment was not considered in the current research project.

The untreated vetiver roots were then also exposed to SCE at two temperatures and pressures (30 and 40°C, 80 and 200 bar). The highest yield obtained was approximately 3.2% at 200 bar and 40°C (refer to Table 2.5). This proved that the SCE method produces higher extraction yields than hydro distillation.

Martinez et al. (2004) then analysed the samples using gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS) techniques (described in section 2. 3). They found 28 compounds being exclusively sesquiterpenes.

By physical analysis, Martinez et al. (2004) concluded that the oil extracts produced by SCE were more viscous indicating that higher molecular weight compounds were present in the sample. However hydro distilled extracts were more volatile. This could explain the increase in yield when looking at SCE compared to hydro distillation. A high fraction of zizanoic acid was found in the vetiver oil produced from Brazil as compared to the commercial standards produced by Haiti, Java and Bourbon. Acids have no sensorial quality and therefore were converted to more valuable khusimol by esterification and chemical reduction.

The commercial standard oils were darker in colour than the oils produced by hydro distillation and oils produced by SCE were even lighter than those produced by hydro distillation. The SCE technique produced a higher amount of acids than the hydro distilled oils. The Brazilian vetiver oil was not suitable for the use in the perfume or food industry without chemical modifications of the oils due to the high amounts of acids present (Martinez et al., 2004), however once the acid content was reduced, the SCE extracts could be used in the perfume industry and the hydro distilled extracts in the food industry.

Talansier et al. (2008) studied the improvement over the conventional process on the quantity and quality of the vetiver extracts recovered by supercritical carbon dioxide extraction (SCE). They also studied the effect of pressure on essential oil yield during SCE. During SCE the yield increased with pressure up to 200 bar, thereafter the yield remained relatively constant with pressure, hence the optimum pressure for SCE was found to be 200 bar at a temperature of 40°C as suggested by Martinez et al. (2004). At this optimum pressure and temperature the SCE yield was 2.9%, however for hydro distillation an extraction yield of only 1.4% was reached (refer to Table 2.5). The main components khusimol, α–vetivone, zizanoic acid and isovalencenol contributed 50% of the composition of the vetiver oil extract.

Talansier et al. (2008) came to very similar conclusions to that of Martinez et al. (2004). This was expected as both investigations used roots from the same company in the São Paulo state in South-eastern Brazil; therefore one can assume that the roots were of the same age and consistency due to common company procedures.

Talansier et al. (2008) also studied the effect of a co-solvent on the SCE process and found that not only did vetiver oil yield increase with increased concentration of ethanol as co–solvent, it increased in a faster time. The best results were obtained for an ethanol concentration of 10% (v/v) giving a yield of 4.7%. The main aim of the work by Talansier et al. (2008) was to study the kinetics of supercritical fluid extraction of vetiver roots.

A similar study was done by Danh et al. (2009) in which they too investigated the effect of pressure as well as the effect of temperature and time in SCE. In order to study the effect of temperature, time and pressure simultaneously on vetiver oil yield the response surface method (RSM) was used. The RSM is a combination of mathematical and statistical techniques used to optimize a parameter influence by many variables.

They found that essential oil yield increased with an increase in pressure up to a pressure of about 190 bar but temperature and time had little effect (refer to Table B.3). The SCE method extracted three times the amount of essential oil extracted by hydro distillation (refer to Table B.2).

Danh et al. (2010) did a similar study but looked at ethanol modified supercritical carbon dioxide extraction in which the ethanol co-solvent is mixed with carbon dioxide solvent prior to heating and entry into the packed column. They studied the effect of the amount of ethanol used and found that the extraction yield also increases with the amount of ethanol co-solvent used up to an optimum point (refer to Table B.5). They found that for SCE with an ethanol co-solvent at 190 bar, 50°C, 15 vol. % ethanol, a 200 min extraction time and a CO_2 flowrate of 2 ml/min (4°C) an extraction yield of 5.9% was achieved. However the extraction yield obtained for regular SCE was approximately 3.74% (refer to Table 2.5). They could therefore conclude that an ethanol co-solvent did improve extraction.

They found that the composition profile of vetiver extracts obtained by SCE and by modified SCE were different to the one obtained by hydro distillation. The yield obtained for SCE was twice that of hydro distillation and the yield obtain by modified SCE was almost triple (refer to Table B.4). The study showed that carbonyl acids were present in higher fractions for SCE extracts as compared to hydro distilled extracts.

Ethanol solvent extraction by Danh et al. (2010) resulted in an extract with a high fraction of a waxy component which was solid at room temperature and large solvent residues which resulted in a yield of 15%. However the sensorial evaluation of the extract indicated that the extract had no valuable application without refining.

Solvent extraction using hexane by Danh et al. (2009) gave a vetiver oil yield of 1.91% (refer to Table 2.5). The hexane extract contained solid particles that were not soluble in hexane at room temperature; these particles were high molecular weight components and hence increased the yield. The extracts from hexane extraction showed a similar chemical profile to that obtained for SCE extracts, however extracts from hydro distillation showed a significantly different chemical profile to both SCE and hexane extraction (refer to Table B.7). This could be due to the fact that hexane and CO_2 are both non-polar solvents and water is a polar solvent.

The extracts obtained by hydro distillation are lower in acid and higher in alcohol content which makes it a more suitable oil for the perfume industry, whereas SCE is a more suitable extraction technique for obtaining products used in the food industry (Danh, 2009). This indicates that different extraction techniques produce oils that are different in composition and therefore the first stage of fractionation is to select an extraction technique that will aid fractionation of the desired components.

The yield obtained by Danh et al. (2010) when using the SCE method was four times greater than that extracted by Danh et al. (2009) when using the same method under similar conditions (refer to Table 2.5). This could be due to the fact that the roots used by Danh et al. (2009) were

only 7 months old whereas the roots used by Danh et al. (2010) were over four years old. This suggests that vetiver oil yield increases with the age of the roots. This appears to contradict the optimum harvesting time of 15 – 18 months for a high vetiver oil yield, however further investigations need to be done into the oil yield for 15 – 18 month old roots.

According to Luque de Castro et al. (1999) the non-conventional techniques are becoming more popular due to the fact that the essential oils extracted from the vetiver grass are used in the perfume industry where better quality essential oil is needed. These non-conventional techniques are said to have a higher selectivity therefore producing better quality essential oils with a higher yield.

In an earlier study done by Aggarwal et al. (1998) it was shown that the sooner the roots undergo extraction after harvesting, the higher the yields of vetiver oil obtained. In the first 12 h of hydro distillation, 96.9% of the total vetiver oil was extracted with majority of the vetiver oil being extracted in the first 2 h. Their study also showed that minimizing the particle sizes of the root from approximately 250 to 50 mm did not increase oil yield.

2. 3 Composition Analysis of Vetiver Essential oil

2.3.1 Gas Chromatography - Mass Spectrometry (GC-MS)

Once the essential oils have been extracted it is necessary to determine which components are present in the oils and their quantity to see if the essential oils are valuable. This analysis can be done using a Gas Chromatography (GC) or a Gas Chromatography-Mass Spectrometry (GC-MS). The GC is used to separate the components of a sample by passing the sample through a long column containing a stationary phase. Depending on the size of column and the type of stationary phase used, each component will be absorbed and desorbed on the surface of the column packing at different rates therefore allowing the components to elute from the column at different times.

However by GC analysis one cannot identify the components eluting from the column, hence the need for mass spectrometry. The eluting components are then sent though the mass spectrometry which contains a library of different components with their mass spectra and therefore with the use of the correct libraries one can determine which component is eluting at which time with a certain degree of certainty.

Essential oils consist of a complex mixture of monoterpene and sesquiterpene hydrocarbons as well as their derivatives which leads to components which exhibit similar mass spectra. Hence an analysis technique is created based on the identification of components by the comparison of their retention data and mass spectra with those found in library data banks as well as in literature. This technique was used for the chemical analysis of the vetiver oil extracted in all the literature sources stated in section 2.2.7.

A specialized library is necessary when dealing with essential oils; this library is known as the Mass Spectra of Flavours and Fragrances of Natural and Synthetic Compounds 1.3. It contains 1813 components with all relevant data for these components. It is also compatible with the commonly used NIST MS interface (Mondello, 2008).

The time at which each component elutes from the GC column is known as the retention time. The most popular way to identify components without using a mass spectrometry is to use the concept of co-chromatography where a standard of the suspected component is injected in the GC and the retention time noted and then compared to the retention times of the components within the sample. The limitations to this method is that one must have an idea of what components are expected in the sample; standards must be available and the column conditions must remain constant for both standard and sample.

From the retention times of the sample and standard, a relative retention time can be calculated. This relative retention time can be documented and used to identify components on other systems with slight differences in temperatures as both standard and sample will be altered in time by the same amount therefore giving similar relative retention times. However this will not work for large temperature differences or for initial acquisition delays (Hochmuth, 2011).

A more accurate identification method is to use retention indices (RI) which is based on the retention times of two standards, one eluting before the component one wishes to identify and one eluting after (refer to Equation 2.1). The standards used are from the alkane series, which are appropriate to use due to the fact that they are non-polar, inert, temperature stable, easily absorbed onto most common stationary phases and can cover a wide range of possible retention times (Hochmuth, 2011 and Van Iterson, 2011). For the analysis of essential oils such as vetiver oil an alkane range of C8-C30 is recommended (Kim et al., 2005).

$$\mathbf{RI_x = 100n_0 + 100\frac{RT_x - RT_{n_0}}{RT_{n_1} - RT_{n_0}}} \qquad \mathbf{2.1}$$

RI = Retention Index
RT = Retention Time
x = Target Component
n_0 = No. of carbon atoms of standard eluting before x
n_1 = No. of carbon atoms of standard eluting after x
Equation 2.1 (Hochmuth, 2011)

One can also use a similar concept known as the Kovats index (Van Iterson, 2011). The Kovats index is the same as the retention index however a logarithmic scale is used instead of a linear scale.

$$\mathbf{KI_x = 100n_0 + 100\frac{LogRT_x - LogRT_{n_0}}{LogRT_{n_1} - LogRT_{n_0}}} \qquad \mathbf{2.2}$$

KI = Kovats Index
RT = Retention Time
x = Target Component
n_0 = No. of carbon atoms of standard eluting before x
n_1 = No. of carbon atoms of standard eluting after x
Equation 2.2 (Hochmuth, 2011)

The Kovats or retention index for any linear alkane will be 100 times the number of carbon atoms in that alkane. Both the retention and the Kovats indices are independent of the system but depend on the stationary phase used within the column of the GC (Hochmuth, 2011). Therefore one can easily identify the compounds of the sample by first calculating the indices of the components within the sample and then comparing them to those tabulated in literature if a constant stationary phase is used. Dimethylpolysiloxane is a commonly used stationary phase

when dealing with essential oils. This is due to the fact that it is inert, non-toxic, inflammable and shows good reproducible performance.

Blatt and Ciola (1991) studied the analysis of vetiver root oil using an online capillary gas chromatography. They used a programmed temperature vaporizing injector between a supercritical carbon dioxide extractor and a capillary column. By doing this they were able to undertake analysis of the vetiver oil by using a small amount of vetiver roots (1 mg). This is convenient for research purposes as harvesting and preparing large amounts of vetiver roots is laborious however one cannot determine yields accurately. At 100 atm they found that a large number of sesquiterpene hydrocarbons were detected with a low amount of sesquiterpene alcohols, aldehydes and ketones as well as no acids. For a pressure of 300 atm there was a lower amount of hydrocarbon sesquiterpenes with a higher amount of all other sesquiterpenes. No tabulated data of retention time or indices was given for Blatt and Ciola (1991) and hence such work is difficult to use as a reference in the future analysis of vetiver oil.

Cazaussus et al. (1988) suggested the use of gas chromatography-tandem mass spectrometry (GC-MS/MS) for the analysis of complex essential oils. The GC-MS/MS system uses an additional mass fragmentation stage (a second quadrupole in a quadrupole instrument) with the GC-MS setup and hence increases selectivity and sensitivity of the device which allows one to distinguish between the isomers and stereo isomers which make up complex essential oils. It was recommended to use a triple quadrupole gas chromatography to get the most accurate results when working with essential oils. The suggested ionization conditions for vetiver essential oil are NCI-OH^-, PCI-NH_4 and PCI-ND_4^+ (Cazaussus et al., 1988).

For the analysis of the vetiver oil a combination of the GC operating conditions shown in Table 2.6 were applied, using a dimethylpolysiloxane column. To identify the components in the vetiver oil extracted for this research the composition data given in appendix B2 as well as retention indices from the Massfinder website (Hochmuth, 2011) were used for comparison.

Table 2.6. Gas chromatography and gas chromatography-mass spectrometry methods for detecting vetiver essential oil

	Gas chromatography		Gas chromatography-mass spectrometry	
	Martinez et al. (2004)	**Danh et al. (2009)**	**Martinez et al. (2004)**	**Danh et al. (2009)**
Type	Varian CP-3380	Shimadzu GC 2010	Agilent 6890 GC	Agilent 6890 GC
Column	Silica capillary	Capillary	Wax infused silica	Capillary
Carrier Gas	Nitrogen	Helium	Helium	Helium
Flow of Carrier Gas/mL.min^{-1}	0.8	1	1.1	1.1
Detector Type	Flame ionization	Flame ionization	Mass Selective	Mass Selective
Injection Volume/µL	1	1	1	1
Injection Type	Split Ratio 1:50	Split Ratio 1:10	Split Ratio	Split Ratio 1:20
Detector Temp./°C	250	250	-	250
Injector Temp./°C	220	220	245	220
Oven Temp.	50-200°C @ 5°C.min^{-1}	50°C for 5min then 50-240°C @3°C.min^{-1} then 240°C for 10min	40-220°C @3°C.min	50°C for 5min then 50-240°C @3°C.min^{-1} then 240°C for 10min
Ionisation Voltage/eV	-	-	70	
Electron multiplier/eV	-	-	1400	
Scan Rate/scan.s^{-1}	-	-	2.96	

2.3.2 Thin Layer Chromatography (TLC)

Thin layer Chromatograph (TLC) is a simple procedure to check how many components are present in a sample. A TLC plate made out of plastic, aluminium card or glass is coated with an absorbent (stationary phase) such as silica or alumina. The TLC plate is then spotted with the sample and allowed to soak in a solvent (mobile phase). The solvent then moves up the plate by capillary action and each component inside the sample on the plate then absorbs and desorbs at different rates (different attraction and solubility) hence when the plate is dried one can see a spotted pattern of how many components there are present in the sample. If a polar stationary phase like silica is used then the more polar components will be more likely to stick to the stationary phase and dispel the mobile phase however the less polar components will travel up further up the plate with the mobile phase.

In terms of the mobile phase a more polar solvent will dispel the solutes from the stationary phase easily therefore allowing all the traces to move further up the plate. For silica as the stationary phase the order of solvents for increasing strength is as follows: perfluoroalkane (weakest), hexane, pentane, carbon tetrachloride, benzene/toluene, dichloromethane, diethyl ether, ethyl acetate, acetonitrile, acetone, 2-propanol/n-butanol, water, methanol, triethylamine, acetic acid, formic acid.

The distance each component travels up the plate is measured as the retention factor and by comparing retention factors to standards one can identify the components (Vogel, 1996). This technique is reserved for natural products consisting of a few components; hence due to the complexity of the vetiver oil composition GC-MS techniques are necessary.

2. 4 Valorisation of the Vetiver Essential Oils

The vetiver essential oil is said to be more valuable from a perfumery point of view if it has a high specific gravity, negative optical rotation, high vetiverol concentration, no residues and a high ester value (Lavania, 2003). By eliminating all residues found in the vetiver oil one allows the oil to be more miscible for blending in perfumes (Danh, 2007). Generally vetiver oil is considered to be of a high quality if the oil is viscous and dark brown in colour.

For the use in the perfume and food industry the vetiver essential oil must be free from all toxins that may cause harm to the consumer. Vetiver roots tend to absorb heavy metals which would be a hazard to the consumer; however it has been proven that due to the high weight of the metals, they stay within the spent roots after extraction (Danh et al., 2010).

The vetiver grass consist of two types of roots (Lavania, 2003), the main smooth roots and the secondary hairy roots. These secondary roots contain unwanted non-polar compounds that reduce the

value of the essential oils. Hence it is necessary to reduce these non-polar compounds by one of the following methods:

- The harvested roots are dried in a cool dry place to allow natural evaporation of the unwanted lighter fraction (Danh, 2007).
- The essential oil extracted in the first 15 - 30 minutes of extraction can be discarded (Lavania, 2003).

One can say that the vetiver oils are valuable for the perfume industry if they contain large amounts of odour influencing alcohols such as khusimol, hence the need to convert undesirable acids and hydrocarbons into valuable alcohol. Therefore other valorisation techniques include the removal of the acids from the vetiver oil or the chemical conversion of the acids into more valuable components such as khusimol (Martinez et al., 2004). The khusimol content is taken as the quality mark of the vetiver oil by some regional producers (Talansier et al., 2008).

Another dominant alcohol found in vetiver oil is vetiverol which gives the oil a cleaner note; therefore for a slightly fruity-woody note one can acetylate this alcohol to vetiveryl acetate (Dowthwaite and Rajani, 2000).

It is recommended to allow the vetiver oil to oxidise in an amber colour bottle for six months to allow the oils to mature into a greener in colour, more valuable product (Danh, 2007).

Fractionation of the vetiver oil extracts is another technique used to increase the value of the essential oils. Fractionation into more specific groups of components such as hydrocarbons, alcohols, ethers, esters etc., can increase the value of the essential oil by allowing the fractions to be used for a more specific function. Fractionation of the essential oils can be achieved by fractional distillation (heat) or by silica solid phase extraction (polarity).

CHAPTER 3: EXPERIMENTAL APPARATI AND OPERATING PROCEDURES

According to literature the highest yields were obtained using supercritical carbon dioxide extraction. However, literature also shows that components in the vetiver oil change according to the extraction method and location of vetiver growth. It is therefore necessary to investigate the yields in terms of quantity and quality for each extraction method when using South African grown vetiver roots. By doing this one can also validate the yields obtained in literature and emphasize the differences in vetiver oils according to location of growth.

3.1. Preparation of Raw Material (Martinez et al., 2004)

After the vetiver roots have been harvested they must be prepared for extraction:

1. The roots are first soaked and washed to remove any unwanted soil contaminants from the ground (refer to Figure 3.1).
2. The roots are then dried in a cool dry place for 2-3 days at room temperature to allow all the low value, non-polar, low boiling components of the oil to evaporate naturally (Danh, 2007).
3. The roots are milled in a knife mill (food processor) which grinds the material into smaller sizes in order to increase the surface area for maximum extraction of the oils. The average particle size was then calculated to be 0.77 mm after milling by a 10 minute vibratory sieve test (refer to Table 3.1).

Table 3.1. Size distribution of vetiver roots (after milling).

Size Range /µm	Percentage
1000 - 2000	60.39
710 - 1000	9.05
500 - 710	8.56
355 - 500	6.60
250 - 355	6.11
<250	9.29

4. The roots are stored in a sealed bag in a freezer at -20°C until extraction to avoid any further loss of volatile component at room temperature.

a **b** **c**

Figure 3.1. Vetiver roots preparation process, a: unwashed, b: washed and c: after milling.

3.2. Evaporation

Apparatus: Roto-evaporator

Evaporation of a solvent is commonly done in a Roto-evap system. The Roto-evap (rotating evaporator) used was a iLMVAC Rodist digital S87. The system consists of two 1 L round bottom flasks, a heating bath and a condenser. The one flask serves as the evaporating or charge vessel and the other is to collect the condensate. The system is also connected to a vacuum pump in order to aid evaporation. The pressure is lowered to below atmospheric to allow for the solvent to boil at a lower temperature making evaporation easier.

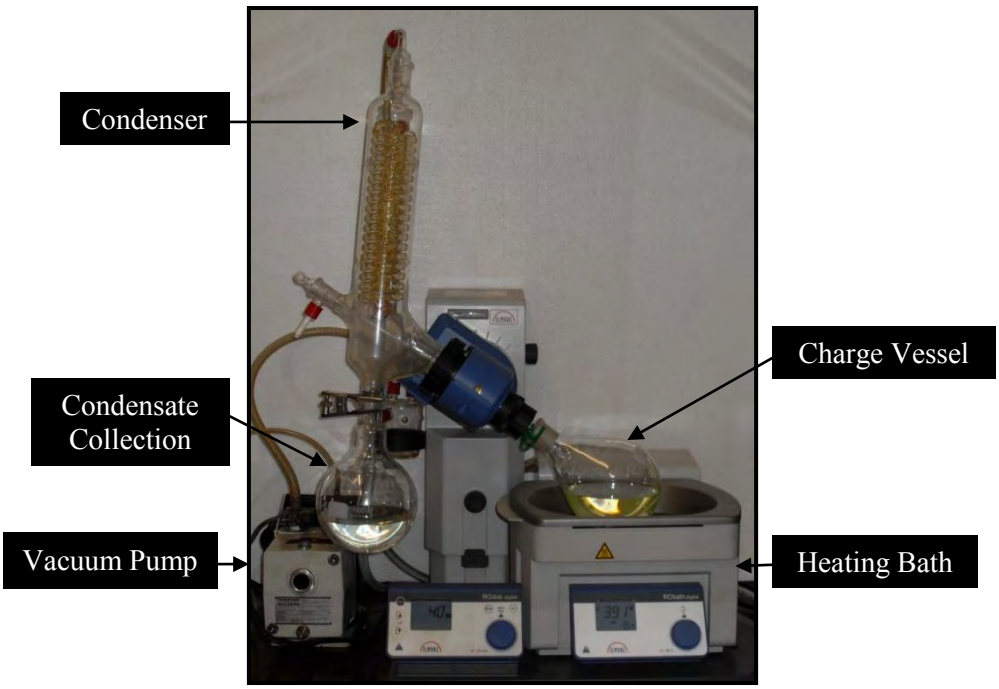


Figure 3.2. Roto-evaporator.

Experimental procedure:

1. Place approximately 30 g vetiver roots inside the charge vessel (1000 ml round bottom flask).
2. Pour 800 ml of distilled water over the roots inside the flask and attach to the right hand side of the roto-evap system.
3. Attach the condensate collection flask to the left hand side of the roto-evap system to collect the condensate and secure with a clamp.
4. Turn the heating bath to the boiling point of the solvent entrained in the extract.
5. Turn on cooling water.
6. Lower the flask containing the extract into the bath.
7. Begin rotation of the flask.
8. Switch on the vacuum pump and ensure the system is sealed.
9. Adjust the pressure (vacuum) to get an even boiling and condensate rate.
10. Allow the solvent to be evaporated.
11. Remove concentrated extract from the flask and place in a fume cupboard for further evaporation if necessary.

3.3. Steam Distillation

Apparatus:
Pilot scale steam distillation unit

Description*:*
The steam distillation unit consists of two parts (refer to Figure 3.3), the vessel which holds the bed of plant material known as the charge vessel and the condenser. The charge vessel is made up of a cylindrical glass body with a 220 mm diameter and a height of 700 mm. It consists of two glass caps which seal the cylinder, the upper cap contains the vapour exit line and the lower cap contains two openings, one for the steam inlet and the other for reflux draining. A plate of wire mesh 215 mm in diameter is used to support the bed of plant material. The plate is connected to a long metal rod which is used to insert the plate into the vessel and then to remove the plant material from the vessel after extraction. The charge vessel is insulated with a 25 mm thick Fiberfrax insulation to reduce heat losses to the environment.

The glass condenser is coiled with the cooling fluid being ambient water on the inside of the coils. The heat transfer area is 1.5 m^2 and the recommended coolant throughput and steam throughput is 1500 $kg.h^{-1}$ and 50 $kg.h^{-1}$ respectively.

The steam enters the bottom of the charge vessel which is packed with plant material. The steam then removes the extracts from the plant material and carries it up and over into the condenser where the vapours are condensed and collected at the bottom of the condenser. The recommended steam flowrate is 4.8 - 9.5 $kg.h^{-1}$ (Talanda, 2005). Both the condenser and charge vessel are made of borosilicate glass with a pressure limit of 1 bar (gauge).

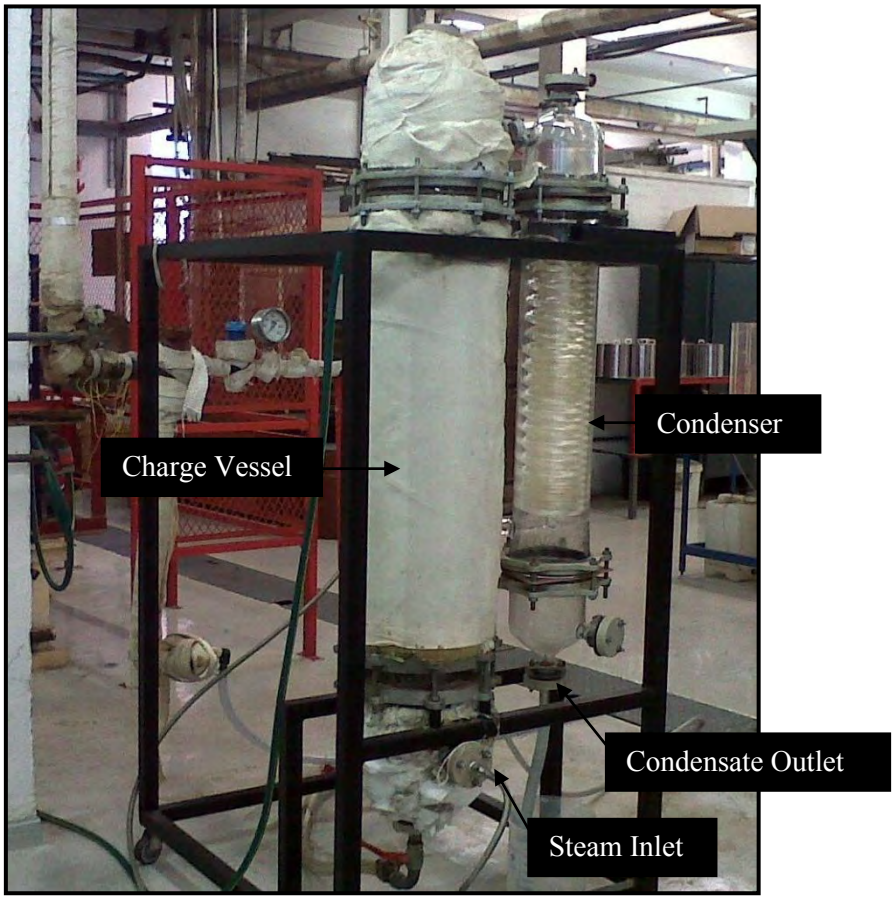


Figure 3.3. Pilot scale steam distillation unit

Experimental procedure:

Load Charge Vessel:

1. Remove bolts on the top section of the charge vessel and on the connecting piece of the charge vessel to the condenser.
2. Remove the top section of the charge vessel.
3. Check the mesh plate is in place at the bottom of the charge vessel with connecting wire for unloading intact.
4. Pour the grass roots into the vessel onto the sieve plate and pack down to secure the bed.
5. Replace the top section of the charge vessel and bolt the system securely.

Start up:

6. Ask workshop staff to start boiler.
7. Wait 30 minutes for steam to build up in the system
8. Place the condensate collecting container (25 L barrel with bottom outlet flow valve) under the condensate outlet point.
9. Close the valve for the reflux outlet.
10. Open the cooling water inlet valve fully.
11. With the valve to the steam distillation unit still closed slowly open the main steam supply to the line.
12. Then slowly open the valve to the unit and regulate the pressure by setting the pressure regulator to the maximum desired pressure. (below 1 bar (gauge))
13. The cooling water and the steam valve to the unit may be set according to the desired condensate rate (Measure using measuring cylinder and stopwatch).

Extraction:

14. Watch the run by continuously replacing the condensate collecting container, once full or to take cuts.

Shut down:

15. Once the specified distillation time is complete close the main steam supply valve, wait a few minutes to clear the line of steam and then close the steam supply valve to the unit.
16. Allow the cooling water to run for a further 30 minutes to clean the condenser of any oils and then close the cooling water supply.
17. Switch off the boiler.
18. One can then collect the reflux by opening the reflux outlet valve.
19. All condensate (vetiver oil and water) and reflux is then collected and the vetiver extracts separated from the water to be weighed for calculation of the yield.
20. Once the unit has cooled completely, one can remove the top section of the charge vessel and remove the spent roots by lifting the sieve plate up and out of the vessel.

3.4. Hydro Distillation

Apparatus:

Clevenger Apparatus

Description:

The Clevenger extraction unit consists of four parts (refer to Figure 3.4), the round bottom flask of 1000 ml capacity, the clevenger arm with 1 ml graduations, the condenser which is coiled with an ethylene glycol water solution inside the coils and a height of 160 mm. The fourth piece is the heating mantle (Glas Col STM1001 230 V/600 W) which is used to heat the round bottom flask during extraction. For full working description on this equipment the reader is referred to section 2.2.1.

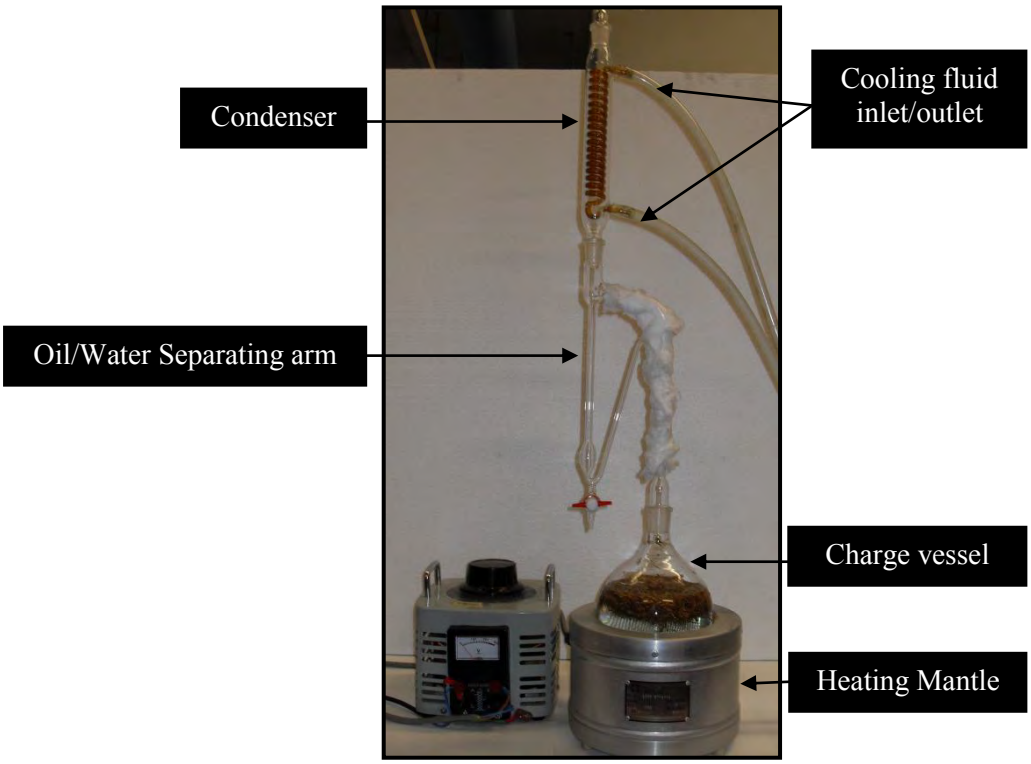


Figure 3.4. Photograph of the Clevenger Apparatus

Experimental Procedure:

1. Place approximately 10 g vetiver roots inside the 1000 ml round bottom flask.
2. Pour 750 ml of distilled water over the roots inside the flask.
3. Place the Clevenger arm onto of the round bottom flask and tighten together.
4. Place whole assembled apparatus in the heating device and support with clamps (Check the tap is closed).
5. Attach the condenser to the top socket of the apparatus.

6. Turn on pump and chilling device for the cooling fluid, the temperature of the cooling fluid is set to 20°C.
7. Turn on the heater on the heating mantle to 85V.
8. Allow to reach to steady state for 1 hour and then extract for the desired extraction time (depending on aim of experiment).
9. After extraction is complete disassemble equipment and collect the concrete (solvent which contains the extracts), open the tap on the Clevenger arm and allow water to drain out first and then collect the oil in a vial.
10. The sample is then weighed to obtain a yield of vetiver oil.

3.5. Solvent Extraction

Apparatus:
Soxhlet Extractor

Description:
The Soxhlet extraction unit consists of four parts (refer to Figure 3.5), the round bottom flask of 250ml capacity and the soxhlet column which consists of a 150 ml bed capacity and a height of 135 mm. The condenser which is coiled with an ethylene glycol water solution inside the coils and has a height of 160 mm and finally the heating mantle (mrc/MNS 250/180W/220V) which is used to heat the round bottom flask. For a full working description on this equipment the reader is referred to section 2.2.2.

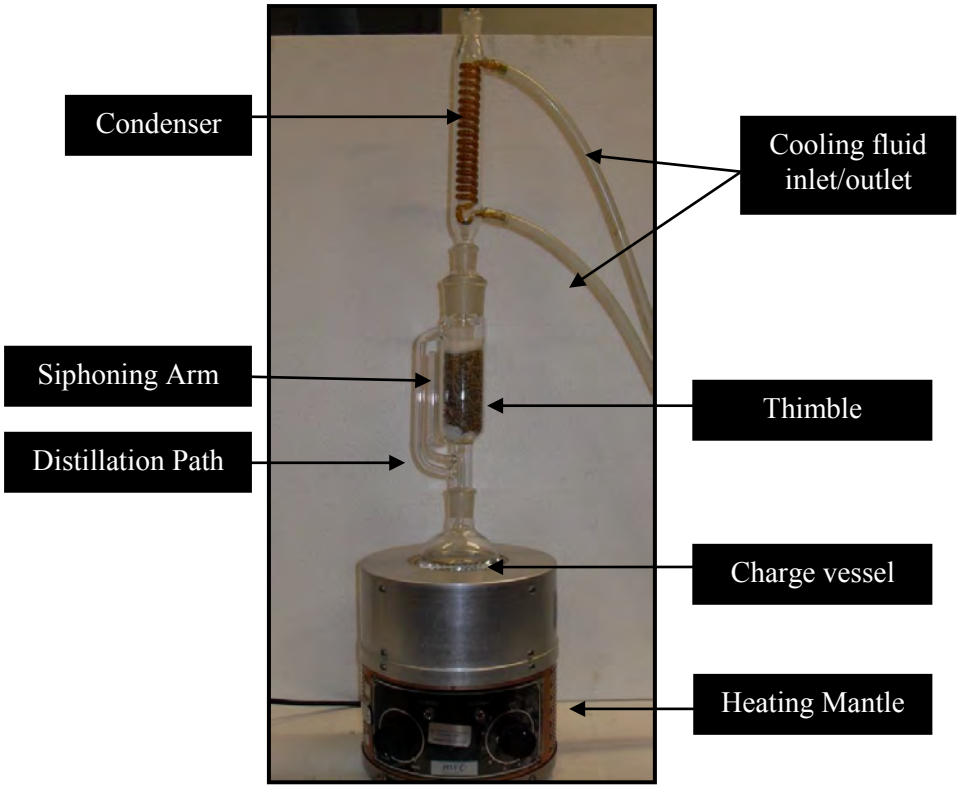


Figure 3.5. Photograph of the Soxhlet Apparatus

Experimental procedure:

1. Pack the soxhlet column with a layer of cotton wool, thereafter approximately 10 g vetiver roots, followed by another layer of cotton wool.
2. Place 150 ml of hexane solvent in the round bottom flask and add a few anti-bumping granules.
3. Place the soxhlet column on top of the round bottom flask and tighten together.
4. Pour an additional 100 ml hexane onto the bed to saturate the bed.
5. Place the lid on the soxhlet apparatus and tighten.
6. Place the assembled apparatus in the heating device and support with clamps.
7. Attach the condenser to the top of the lid of the apparatus.
8. Turn on the pump and chilling device for the cooling fluid; the temperature of the cooling fluid is set to 20°C.
9. Turn on the heater on the heating mantle to a setting of 3 (Scale: 1-10 / 180W / 220V).
10. Allow the system to reach steady state for approximately 10 minutes and then extract for the desired extraction time (depending on aim of experiment).
11. After extraction is complete dissemble equipment and collect the concrete.
12. The solvent is then evaporated in a rota-evaporator (refer to section 3.2) and then placed in a fume cupboard to remove any residual hexane left in the extract.
13. The sample is then weighed to obtain the yield of vetiver oil.

3.6. Supercritical Carbon Dioxide Extraction

Apparatus:
Supercritical Fluid Extraction Setup

Description:
The supercritical fluid extraction setup is shown in the process flow diagram below. The supercritical fluid used was carbon dioxide; hence a technical grade 17 m^3 carbon dioxide cylinder, supplied by Afrox was attached to the inlet of the setup.

The main challenge faced during the design of the supercritical carbon dioxide setup was the many phase changes which CO_2 undergoes during the process beginning as a supply from the cylinder to the exit of the extraction setup. The carbon dioxide enters the extraction setup from a cylinder as a gas at approximately 80 bar, it is then cooled to -15°C at which it becomes a liquid to be pumped by the HPLC pump and finally it is heated to 40°C where it is a supercritical fluid.

All the phases have different densities and hence when looking at the optimum velocity for flow through a pipe as recommended by Coulson and Richardson (2006), a tube size of 0.3175 cm was chosen. This tube size gives an inner diameter greater than the maximum inner diameter calculated according to all the phase changes occurring in the system (refer to Table C.1).

Another challenge was the high working pressure of between 80 -200 bar. All valves and fittings were stainless steel high pressure parts supplied by Swagelok. A 2 cm diameter by 31.8 cm length bed was selected (based on Martinez et al., 2004) to minimise the pressure effects on the ends of the extraction vessel as the change in diameter between the tubing and vessel inner diameter is minimised. Due to the long bed length the pressure drop over the bed was expected to increase; however the pressure drop over the bed was considered to be negligible due to the high porosity of the bed ($\varepsilon = 0.94$; $\rho_p = 1\,560$ kg.m^{-3} (Talansier et al., 2008) and $\rho_b = 92.5$ kg.m^{-3}).

The necessary extraction vessel thickness was calculated as 1.73 mm; this was based on the design pressure, material stress and inner diameter of the extraction vessel (refer to Appendix C). However for the two ends of the vessel a non- flanged flat end was used and hence the thickness of the vessel was increased to 2 cm to allow for sufficient surface area for the bolted circumference. Thickness of the flat ends was calculated to be 9.98 mm and hence a thickness of 10 mm was used on either end (refer to Appendix C).

The extraction vessel was designed and built in the workshop; a 6 cm by 31.38 cm cylindrical stainless steel bullet was used. A 2 cm diameter was drilled through the centre of the bullet and then two non-flanged flat ends were constructed with 6, 6 mm bolts on either end (refer to Supercritical Carbon dioxide Extraction Cell drawing, Appendix C).

Within the setup there is a cooling and a heating stage, the cooling setup consists of a temperature controller (Grant GR150 230 V/22 VA/ 50 Hz) and a chiller (Grant C2G 220-240 Vav/400 W/50-60 Hz) both inside a cooling bath (H: 20 cm/ W: 34 cm/ L: 42 cm). The heating setup consists of a temperature controller (Grant GD120 220-240 V/1.5 KW/ 50 Hz) inside a heating bath (H: 50 cm/ W: 30 cm/ L: 60 cm) and a temperature probe (WIKA with a Zenith display) inserted next to the extraction vessel to verify the temperature within the large bath. Both stages contain a heat transfer coil of required length for maximum heat transfer (refer to Appendix C).

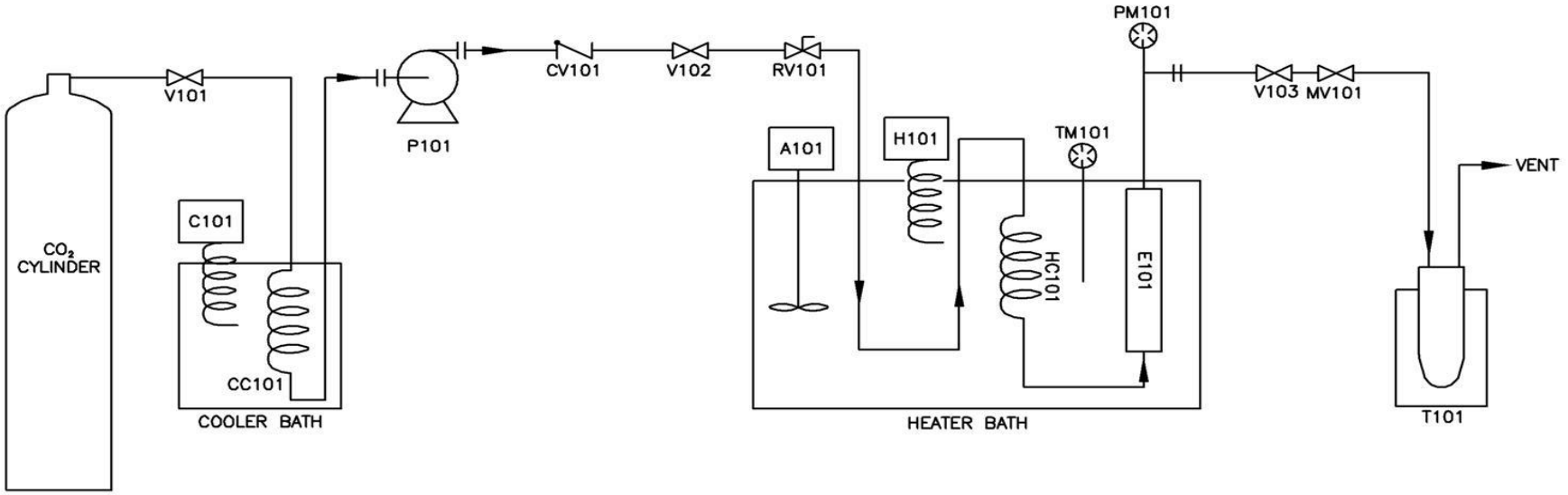
A Beckman Model 110A HPLC Pump (lower limit set: 27579 kPa, upper limit set: 41368 kPa) was used to increase the pressure of the CO_2 entering the system. This is a liquid pump and hence the need to cool the pressurized CO_2 coming from the cylinder to convert it to a liquid state. According to the phase diagram for CO_2 (refer to Figure F.1), it becomes a liquid at approximately 60 bar (pressure delivered by the cylinder) and approximately 5°C.

With time the pump begins to heat up due to the moving piston and hence the liquid CO_2 starts to become a gas again and the pumping fails. Due to the fact that the cylinder pressure cannot be increased the only parameter that could be adjusted to move the CO_2 more into the liquid region was to decrease the temperature of the CO_2 entering the pump. This compensates for the heat produced during the mechanical operation of the pump.

A peltier plate (Laird CP14, 127, 06, L1, W4.5/ 15.4 V/51.4 W/6 A) was also installed on the pump face to cool the fluid as it moves through the pump. The peltier required a large heat sink to remove the heat from the hot side of the plate to allow the cooling side to get to a temperature of approximately 5°C.

A hazard and operability study was performed on the system (refer to Table C. 3); the main hazard was a build-up of pressure within the system. This would be caused by a blockage anywhere after the pump; hence the installation of a pressure relief valve (Swagelok SS-4R3A-SETE) set to 200 bar. The system was also flushed regularly with hexane in order to make sure there were no blockages.

For a full working description on this equipment the reader is referred to section 2.2.5.



Number	Item
V101-3	Stopping valves
C101	Cooler
CC101	Cooling coils
P101	HPLC pump
CV101	Non-return valve
RV101	Pressure relief valve
A101	Agitator
H101	Heater
HC101	Heating coils
TM101	Temperature probe
E101	Extraction vessel
PM101	Pressure gauge
MV101	Metering valve
T101	Cold trap

UNIVERSITY OF KWAZULU NATAL
SCHOOL OF CHEMICAL ENGINEERING

SUPERCRITICAL CARBON DIOXIDE
EXTRACTION SET UP
PFD

DRAWN BY: Bianca Leite
DATE: 30 August 2011

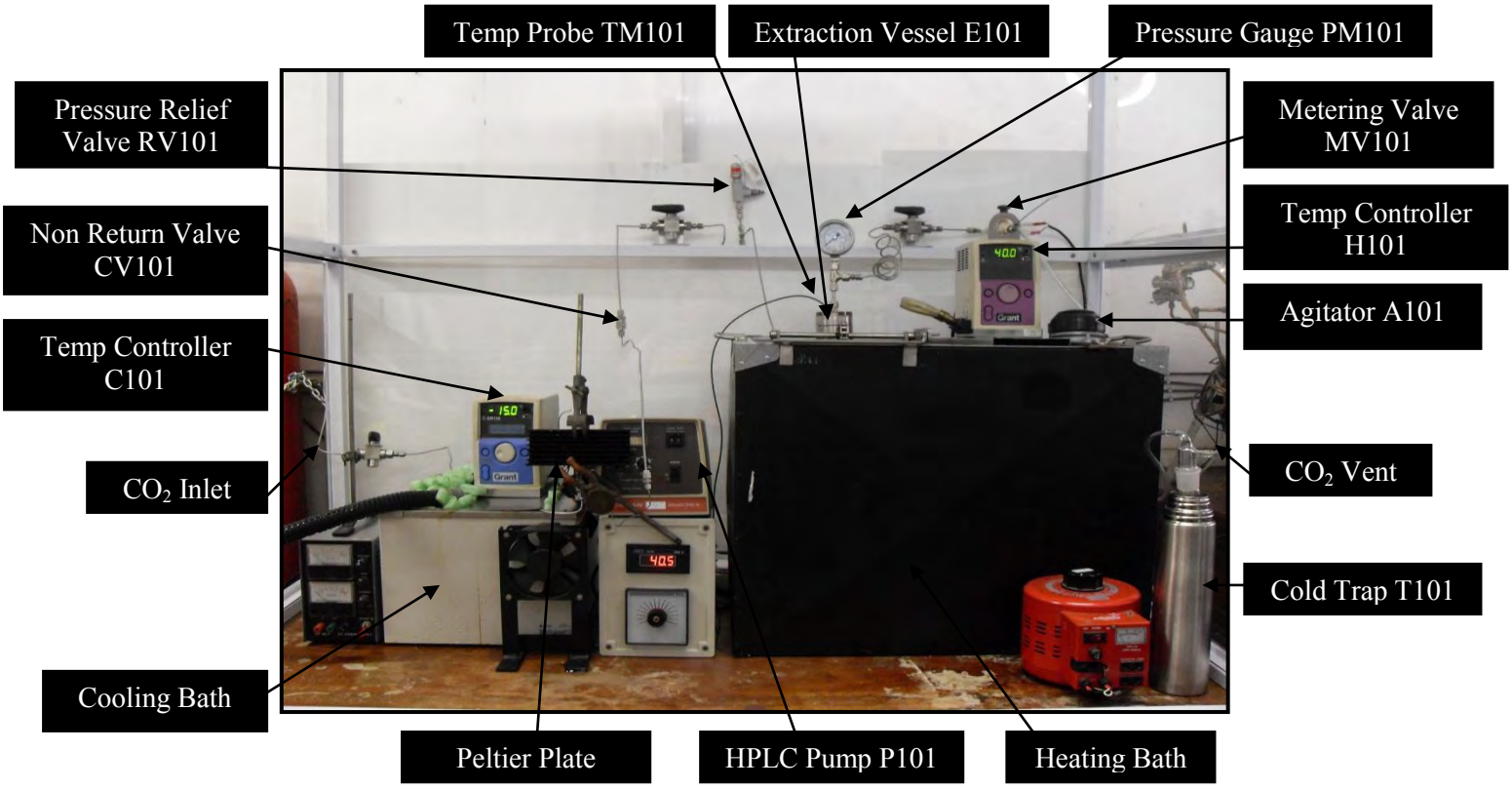


Figure 3.6. Photograph of the Supercritical Extraction apparatus

Experimental Procedure:

Start up:

1) Fill the extraction vessel (E101 / V=100 ml) with prepared vetiver roots and plug the bed on both sides with glass wool and the stainless steel filters (250 µm sieve size).
2) Fill the cold trap with chilled ethanol (-20 to -5 °C).
3) Seal the extraction vessel (E101) by bolting the lid closed.
4) Lower the extractor into the heating bath.
5) Set the heater (H101) to the desired process temperature and switch on the agitator (A101).
6) Turn on cooler and chilling device (C101) for the cooling fluid (ethylene glycol water solution), set the temperature of the cooling fluid to -15°C.
7) Once the heating and cooling baths have reached the desired temperatures, open valves V101-103 fully and close the metering valve (MV101).

Static stage:

8) The CO_2 cylinder is opened to its maximum pressure (approximately 80 bar).
9) Set the pump (P101) flowrate to 9.9 $ml.min^{-1}$, (maximum flowrate on the Beckman 110A) and the pressure to the desired operating pressure. P101 is then switched on.
10) Allow the system to reach steady state by monitoring the pressure on the pressure gauge (PM101) until the system is full of fluid and the pressure begins to rise.

Dynamic stage:

11) Slowly expand the supercritical CO_2 by opening MV101 until the pressure is constant at the desired operating pressure.
12) The gaseous CO_2 with vetiver oil extract flows into the glass trap (T101) where the extract is collected.
13) This dynamic stage is allowed to continue for the desired extraction time.

Shut down:

14) After extraction is complete switch off P101 and close the CO_2 cylinder.
15) Any remaining pressure in the system is released through MV101.
16) The CO_2 inlet line to the pump is then removed and connected to the hexane tank. Hexane is then pumped through the system to remove any extract collected along the exiting tubing.
17) Close valves V101-3 and MV101.
18) The essential oil and hexane solution is then removed from T101, evaporated in a rota-evaporator (refer to section 3.2) and then placed in a fume cupboard to remove any residual hexane left in the extract.
19) The sample is then weighed to obtain the yield of vetiver oil.

3.7. Composition Analysis of the Extracted Material

Apparatus:

Table 3.2. Gas-chromatography method for chemical analysis of the vetiver oil samples.

	Method
Type	Shimadzu GC 2010
Column	ZB-1HT (30 m L x 0.25 mm ID x 0.25 µm film thickness) 100% Dimethylpolysiloxane
Carrier Gas	Nitrogen
Linear Velocity/ mL.min^{-1}	25
Detector Type	Flame ionization
Injection Volume/µL	0.5
Injection Type	Split Ratio 1:50
Detector Temp./°C	250
Injector Temp./°C	220
Oven Temp.	50-200°C @ 5°C.min^{-1}
Method Based on.	Martinez et al. (2004)
Comparison of.	Retention Indices

Procedure:

Preparation:

1. Turn on the carrier gas flow (refer to Table 3.2).
2. Set GC method on the computer (refer to Table 3.2) and allow the settings to stabilise by reaching the required temperatures and flowrates for analysis.
3. Inject 1µL n-alkane mixture standard (C_7-C_{30}) into the GC.
4. Record n-alkane retention times.

Analysis:

5. Mix 1 drop of the vetiver oil sample of unknown composition in 5 ml of n-hexane and mix until dissolved, heat if necessary.
6. Inject 1 µL of the unknown sample into the GC.
7. Record retention times for the unknown peaks.

Identification:

8. Calculate the retention indices for all the unknowns using Equation 2.1.
9. Identify the components by comparing the retention indices to those found in literature.
   *Retention indices are reproducible for a typical system with the same column stationary phase with a certainty of +/-5RI. (Hochmuth, 2011).

CHAPTER 4: RESULTS

4.1. Dry Root Mass Yields

Figure 4. 1. Vetiver roots extracted from the Newlands Mashu site (left) and vetiver grass according to literature (right) (Truong et al., 2008).

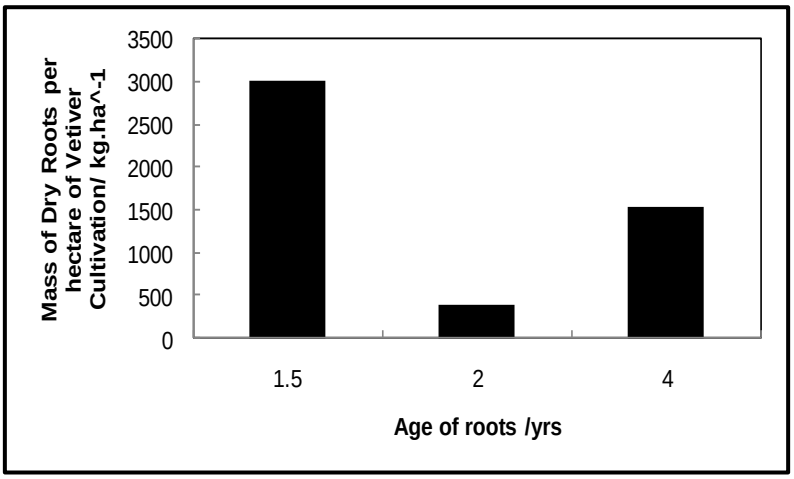


Figure 4.2. Harvesting yield of vetiver roots, Roots of age 2 and 4 years were harvested from the Mashu Newlands site, the yield stated for the 1.5 year old roots was from a site in India (NEDFi, 2005).

4.2. Distillation Results

Table 4. 1. Yields obtained by Hydro distillation of vetiver oil (4 years old roots).

Dry weight of roots/ g	Volume of Water/ ml	Extraction time/ h	Yield of vetiver oil /wt% dry roots
12.5	750	1	0.044 ± 0.028
12.5	750	2	0.077 ± 0.067
12.5	750	4	0.124 ± 0.056
12.5	750	8	0.198 ± 0.058
12.5	750	16	0.307 ± 0.099
12.5	750	24	0.391 ± 0.122

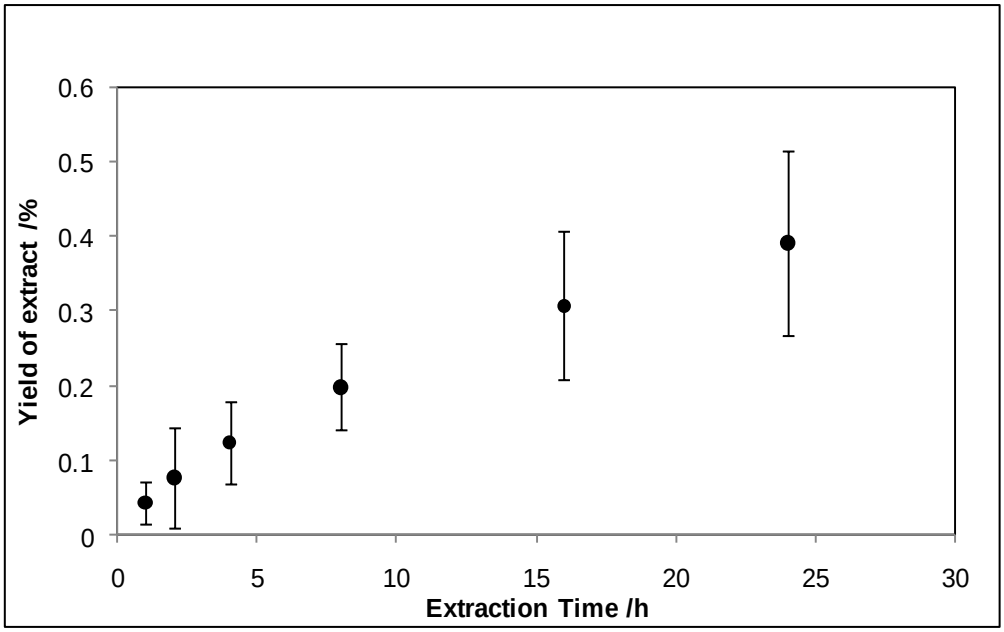


Figure 4.3. Yield of vetiver oil extracted by Hydro distillation in a Clevenger apparatus at various time increments (data with error bars).

4.3. Solvent Extraction Results

Table 4.2. Yields obtained by solvent extraction of vetiver oil (4 year old roots).

Scale	Dry weight of roots/ g	Volume of Hexane/ ml	Extraction time/ h	Yield of vetiver oil /wt.% dry roots
Large	223.6	5000	12	1.87
Small	12.5	250	1	0.84 ± 0.28
Small	12.5	250	2	1.24 ± 0.17
Small	12.5	250	4	1.66 ± 0.10
Small	12.5	250	8	1.66 ± 0.09
Small	12.5	250	12	2.07 ± 0.03

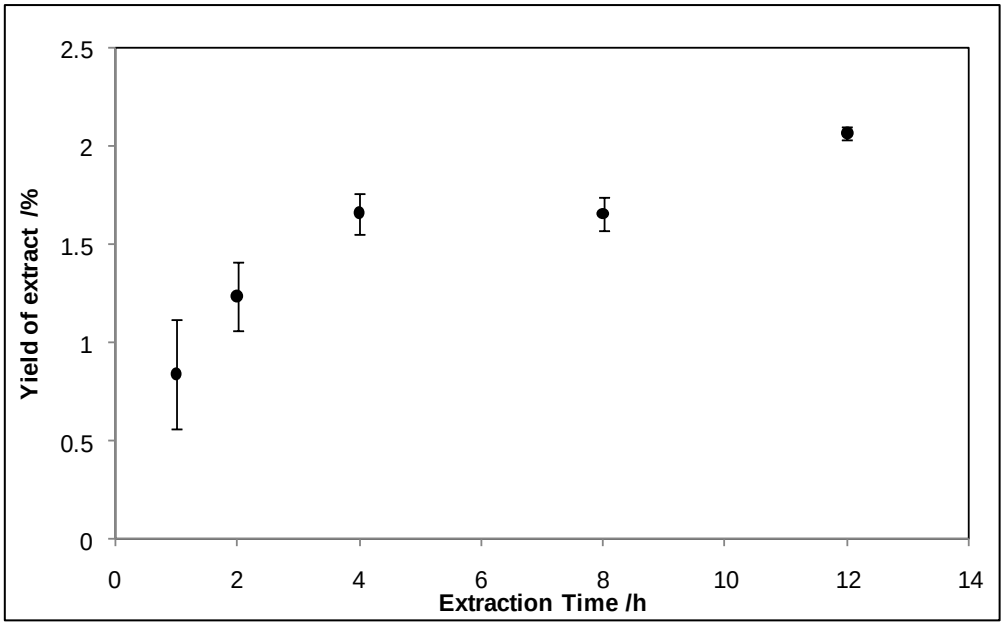


Figure 4.4. Yield of vetiver oil extracted by Solvent (hexane) extraction as a function of extraction time (data with error bars).

4.4. Supercritical Carbon Dioxide Extraction Results

Table 4.3. Yields obtained by supercritical CO_2 extraction of vetiver oil (4 year old roots).

Dry weight of roots/ g	Temp / °C	Pressure / bar (gauge)	HPLC Pump setting for CO_2 Flowrate / ml.min-1	Extraction time/ min		Yield of vetiver oil /wt.% dry
				Static	Dynamic	
10.5	40 ± 0.5	80 ± 5	9.9	10	60	2.3 ± 0.54
10.5	40 ± 0.5	180 ± 5	9.9	20	60	2.26

4.5. Effect of Age of Vetiver Roots

Table 4.4. Effect of the age of the vetiver roots on vetiver oil yield for solvent extraction.

Age of vetiver grass /yr	Dry weight of roots/ g	Volume of Hexane/ ml	Extraction time/ h	Yield of vetiver oil /wt.% dry roots
2	12.5	250	8	2.7 ± 0.18
4	12.5	250	8	1.66 ± 0.09

Table 4.5. Effect of the age of the vetiver roots on vetiver oil yield for hydro distillation.

Age of vetiver grass /yr	Dry weight of roots/ g	Volume of Distilled Water/ ml	Extraction time/ h	Yield of vetiver oil /wt.% dry roots
2	12.5	750	8	1.44 ± 0.48
4	12.5	750	8	0.20 ± 0.06

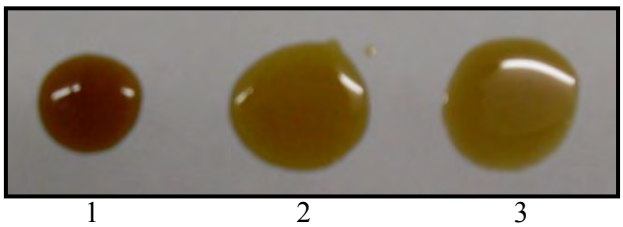
Table 4.6. Effect of the age of the vetiver roots on vetiver oil yield for supercritical CO_2 extraction.

Age of vetiver grass /yr	Dry weight of roots/ g	Temperature / °C	Pressure / bar (gauge)	Mass of CO_2 used /g	Extraction time/ min		Yield of vetiver oil /wt.% dry roots
					Static	Dynamic	
2	10.5	40 ± 0.5	80 ± 5	613	10	60	2.58
4	10.5	40 ± 0.5	80 ± 5	613	10	60	2.30 ± 0.54

4.6. Composition Analysis Results

Table 4.7. Physical properties of experimental and standard vetiver oil.

	Experimental	Standard - India	Standard - Indonesia
Refractive index @ 20°C	1.515 ± 0.000768	1.508 ± 0.000068	1.516 ± 0.000097



1 2 3

Figure 4.5. Vetiver oil, 1: Experimental, 2: India, 3: Indonesia.

Table 4.8. Composition analysis results showing the comparison of the vetiver oil obtained in this work to the vetiver oils purchased.

Identification	% Area*			Calculated Retention Index	Literature Retention Index**
	Experimental***	India	Indonesia		
α- Duprezianene			4.49	1387	1388
β- Funebrene		11.72	3.15	1416-1417	1418
β-copaene			2.44	1436	1430
Pre-zizaene			4.83	1450	1452
α-Patchoulene		15.74		1463	1467
α-amorphene			5.90	1474-1478	1477
Cis-eudesma-6,11-diene			4.89	1484	1484
δ-Cadinene			5.74	1519	1520
Elemol		20.37	5.71	1540-1545	1541
β-vetivenene	2.93	10.46	3.42	1550-1551	1552
Sphathulenol	2.89		4.00	1575	1572
Pogostol/Valerianol	5.70		4.11	1643-1644	1647
7-epi-α-Eudesmol	2.86		2.88	1654	1653
Khusinol	3.64		2.98	1663	1668
Vetiselinenol	4.66		2.66	1702-1709	1709
Khusimol	9.33	8.35	8.00	1720-1721	1720
Isovalencenol		5.20		1778	1779
Nootkatone	22.64		8.77	1784	1782

* Total percentage area excludes hexane solvent/ all unidentified areas are not stated.

** Hochmuth , 2011 : Terpenoids Library

*** The experimental results stated above were taken from the 24 hr hydro distilled extract.

Table 4.9. Composition analysis results for vetiver extracts.

Identification	% Area*						Calculated Retention Index	Literature Retention Index**
	Hydro distilled		Solvent Extraction		SCE			
	4 yr Roots	2 yr Roots	4 yr Roots	2 yr Roots	4 yr Roots	2 yr Roots		
α-amorphene		1.53					1473	1477
β-vetivenene	2.93	2.21					1549-1550	1552
Sphathulenol	2.89	1.75					1574	1572
Pogostol/Valerianol	5.70	8.30		3.58			1644	1647
7-epi-α-Eudesmol	2.86	2.33		1.87			1654	1653
Khusinol	3.64	3.90	2.65	2.00			1663-1664	1668
Eudesma-4(15),7-dien-1β-ol		2.55					1676	1671
Vetiselinenol	4.66	5.54	5.36	4.65	1.61	2.29	1702-1707	1709
Khusimol	9.33	15.05	7.04	11.22	5.01	6.73	1716-1722	1720
Nootkatone	22.64	10.40			9.55	15.66	1782-1787	1782
Zizanoic acid			38.75	30.38	1.56	1.64	1790-1796	1798
α-vetivone		2.39	5.29	2.98	1.40	1.48	1813-1827	1821

* Total percentage area excludes hexane solvent/ all unidentified areas are not stated.
** Hochmuth , 2011 : Terpenoids Library
Hydro distilled: 4 yrs = 24 hr extraction and 2 yrs = 8 hr extraction
Solvent extraction: 4 yrs and 2 yrs = 8 hr extraction
SCE: Supercritical extraction - 80 bar/ 40°C

CHAPTER 5: DISCUSSION

5.1. Harvesting and Preparation

Vetiver grass was supplied by the eThekwini Water and Sanitation Department of the Durban Municipality. The location of the vetiver grass is Newlands Mashu; this is a municipality site which contains a wastewater collection point for the local communities as well as many types of crops which are used mainly for research purposes. The vetiver grass supplied was *Vetiveria zizanioides* from a rain fed ground source with the roots being either 2 or 4 years of age.

On the visit to the site for the collection of vetiver roots, it was immediately seen that the vetiver roots were not as long and intertwined as suggested in literature (refer to Figure 4.1). This could be due to the hard clay soil conditions present at the Mashu Newlands plant or due to the fact that there are water and nutrients sources readily available for the roots. The Vetiver network states that when the roots are exposed to easily accessible water and nutrient sources there is no need for the plant to grow to find this source and therefore the roots will be shorter in depth and less intertwined (Grimshaw and Dafform, Vetiver Network).

Based on the roots harvested from the Mashu Newlands Municipality site in Durban, a dry root mass of 384 kg per hectare of vetiver cultivation was recorded using a sample of 2 year old roots as well as a dry root mass of 1 536 kg per hectare vetiver cultivation using a sample of 4 year old roots (refer to Figure 4.2). These values of dry root mass are very low compared to the 3000 kg of dry roots per hectare of vetiver cultivation (1.5 years old) that is obtained at a plant in India (NEDFi, 2005). Another reason for low yields could be due to loss of roots during harvesting and hence it is recommended to use bags of soil for planting instead of planting straight into the ground.

As discussed in section 2.2.7 no chemical pre-treatment of the roots was necessary, however all vetiver root samples collected underwent the preparation steps described in section 3.1. The harvesting of the vetiver roots was a very laborious process as the roots grow deep and intertwined into the ground and therefore care had to be taken when removing the roots from the soil to minimize the loss of roots left behind. Washing of the roots was also very laborious as the soil conditions at Mashu Newlands were hard and clay like; hence the roots had to be soaked prior to rinsing. Again care was taken not to lose roots by washing them down the drain.

Once the roots were cleaned and dried a strong woody vetiver aroma was already noted. It was then necessary to grind the vetiver roots into smaller pieces in order to increase the surface area for extraction. The vetiver roots were ground in a knife mill in small batches to allow the knife mill to

cool in-between operations. This was done to decrease the effect of heat degradation on the vetiver roots.

The moisture loss from vetiver roots was investigated when 10 g of dry vetiver were placed in an oven at 40°C. This showed that over 2-6 days the roots lost 0.4 g of mass from 10 g of roots. This may seem insignificant however when working with such small yields of oil this amount does become significant therefore after preparation of the vetiver roots (refer to section 3.1) it was necessary to store the roots in a cold location such as a freezer (approx. -20°C) to prevent any mass (which could possibly be essential oil) loss during storage.

The yield of vetiver oil is defined as the mass of vetiver extract divided by the mass of dry vetiver roots used to obtain that mass of extract and then converted to a percentage .When determining the yield of oil from the vetiver roots there were some uncertainties such as left over solvent in the vetiver oil extract after evaporation as well as due to uneven distribution of oil within the roots. To minimize these uncertainties, all experimental runs were repeated a minimum of three times to calculate a standard deviation and hence prove reproducibilty. A rate of hexane evaporation curve was also obtained and it was observed that the extract reached a steady mass (implying all solvent has been evaporated) at approximately 14 hours; hence all extracts were subjected to at least 14 hours of evaporation (refer to Appendix E).

5.2. Distillation

Initially some trial hydro distillation runs were executed in the roto-evaporator (refer to section 3.2). The vetiver grass was packed into the rotating flask/charge vessel and this was filled with distilled water. The water was allowed to boil and then condense into the condensing flask. Two runs were undertaken both at the boiling point of water; however in one run the system was under vacuum and in the other it was at atmospheric pressure. No recycle of condensate was used, hence the distillation times were short. In the vacuum distillation run, a large amount of water loss was observed which was due to an inefficient condenser.

Overall the runs where unsuccessful because there was no visible vetiver oil yield in the condensate, it was a clear liquid with a slight vetiver odour. This could be due the fact that the sesquiterpene components that are known to make up the vetiver oil boil in the 200+°C range (refer to section 2.1.4), hence the oils could not vaporize and condense at the temperatures provided by the roto-evaporator. It is possible that the vetiver extract was still sitting in the boiling flask on the spent roots as there was a pungent vetiver odour coming from the spent roots.

A steam distillation run was then carried out using a pilot size steam distillation unit in the laboratory at the School of Chemical Engineering at the University of KwaZulu-Natal. The unit consisted of a 40 L charge vessel with a steam connection at the bottom and a connection into an adjacent condenser

at the top (refer to section 3.3). The charge vessel was packed with 827.5 g prepared vetiver root material and steam was passed over the packed bed at 80 kPa (gauge) (± 116°C). A pressure of 80 kPa (gauge) was chosen according to the restrictions of the glass pilot plant which cannot take pressures exceeding 100 kPa (gauge). In an extraction time of 5 h, 36 L of condensate was collected.

After collection of the condensate one could visibly see a shimmery layer of oil however due to the large amount of condensate present and such a small amount of vetiver oil, this extract could not be recovered during decanting (a noticed amount of droplets adhered to the sides of the containers and therefore could not be quantified). Another possible reason for the low vetiver oil yield could be due to the fact that the system pressure was too low. A higher pressure is needed to rupture the oil cells to extract high boiling sesquiterpenes (refer to section 2.2.1). In fact, Dowthwaite and Rajani (2000) recommend a 300 kPa (gauge) steam pressure to extract sufficient vetiver oils using steam distillation which was not possible with the equipment limitations.

For collection of vetiver oil during distillation it is recommended to include an oil/water separation system (decanter arm) by the condensate outlet point where water can be drained off the bottom and vetiver oil easily recovered. Such modifications were not possible on the equipment available.

Due to the above limitations, a laboratory scale clevenger apparatus was made by the local glass blower (refer to section 2.2.1), which included an oil/water separating arm. In order to aid the removal of vetiver oil extract from the arm of the apparatus, 2 ml of hexane (selected due to its immiscibility in water) was added to the arm. The hexane was then evaporated in a fume cupboard.

The results obtained from the analysis of the hydro distillation experiments in a clevenger apparatus shown in Figure 4.3 indicate that the vetiver oil yield increases linearly with time. For a 16 hr extraction time the yield obtained by analysis was 0.307 ± 0.099% which is significantly lower than the yields reported in literature of approximately 1.8% for a 16 hr extraction. Possible reasons for the variation could be due to losses of extract during removal from the arm; the components within the oil extracted by hydro distillation of the locally grow vetiver grass could be much lighter than those roots grown in other areas such as Brazil where most of the literature sources obtained their roots or the higher molecular weight component were not removed at the boiling temperature of water.

5.3. Solvent Extraction

For the solvent extraction experiments, a large scale (5 000 mL solvent recycle volume) soxhlet apparatus located at the University of KwaZulu-Natal Chemistry department was initially used. Hexane was selected as the solvent as advised by literature as well as by the hexane evaporation test undertaken (refer to Appendix E). A vetiver oil yield of 1.87% was obtained (refer to Table 4.2). The extract was amber brown in colour as desired however the odour contained strong traces of hexane, indicating that extracted vetiver oils by solvent extraction are not valuable for the perfume industry.

A smaller scale (250 mL solvent recycle volume) soxhlet apparatus was set up in the Thermodynamics laboratory in the School of Chemical Engineering at the University of KwaZulu-Natal. It was necessary to have a smaller scale unit because of the large amount of labour that goes into obtaining and preparing the root samples.

Investigations into the effect of extraction time and boiling rate within the soxhlet apparatus were then undertaken. The yield of vetiver oil increased with time reaching a maximum value of 2.07% for an extraction time of 12 hours. There appears to be distinct periods in which extraction is taking place (refer to Figure 4.4); between 4 – 8 hours no extraction occurred whereas an increase of approximately 1.66% yield of vetiver extract was observed between 0 – 4 hours and a further 0.4% yield of vetiver extract was observed between 8 – 12 hours.

For comparison with literature one looks at a 5 hour extraction time which gave a yield of aproximately 1.6% which is slightly lower than the 1.91% yield that Danh et al. 2009 obtained for a 5 hour run.

Experiments at different boiling rates of the solvent showed that by increasing the boiling rate hence recycle rate of hexane within the soxhlet apparatus one can obtain higher yields (an increase in recycle time from 6.5 minutes to 13 minutes showed a $\pm$ 0.4% increase in vetiver oil yield). However by increasing the boiling rate one increases the temperature within the vetiver root bed which in turn could damage thermolabile components within the oil and the process would become more energy intensive. Hence all runs were carried out at the lower boiling rate.

5.4. Supercritical Carbon Dioxide Extraction (SCE)

A significant portion of the research project focused on the design and commissioning of a supercritical carbon dioxide extraction unit. Based on the units described in literature, a design was put together (refer to Appendix C) with an operating pressure and temperature of 220 bar and 50 °C respectively.

When operating the SCE setup a few challenges were faced. During the dynamic stage of operation the metering valve needed to be opened slightly to be able to maintain the pressure at the desired operating pressure and at the same time allow for a continuous flow. Due to the phase change that occurred at the exit of the system as the pressure was throttled from a high pressure to atmospheric pressure the CO_2 takes in energy causing the fluid to freeze and hence block the exit line. This causes the exit flow and the pressure within the system to fluctuate slightly and hence the metering valve had to be continuously adjusted manually during dynamic operation. A heating wire was then installed to heat the metering valve and exit line to provide the energy needed for this phase change and hence prevent large fluctuations in flow and pressure.

After the setup and testing of the SCE unit was complete, trial experiments were performed. According to literature (refer to section 2.2.7) 60 minutes of dynamic extraction is sufficient to remove the majority of the vetiver oil from the roots and hence two extraction scenarios were tested; 80 bar and 40°C and 180 bar and 40°C both with a 60 minute dynamic extraction time.

The temperature was kept as close to the critical temperature as possible to decrease the adverse effects on the thermo labile components within the vetiver oil. One high and one low pressure run was chosen to see the effect of pressure on extraction yield. At 80 bar and 40°C a yield of 2.3 $\pm$ 0.54 % was achieved and by increasing the operating pressure to 180 bar a yield of 2.26% was achieved; showing that by increasing the pressure more energy is used but there is no increase in yield when keeping all other operating conditions constant (refer to Table 4.3).

Research shows that supercritical carbon dioxide extraction (SCE) of vetiver oil produces the highest yields ranging from 2.9 - 3.74% when using the recommended parameters of 190 bar and 50°C (refer to section 2.2.7). The yield obtained in this work is lower than data reported in literature due to the lower operating temperature and pressure however SCE gives a higher yield than the other extraction methods tested in this project.

5.5. Effect of Age of Vetiver Roots on Vetiver Oil Yields

According to literature the optimum time to harvest these root is 18 months (NEDFi, 2005) hence one needs to investigate the yield and quality of the vetiver oils extracted from the vetiver grass at different ages.

Vetiver oil was extracted from roots of age 2 years and 4 years. Studies showed that the younger roots give higher vetiver oil yields for all three of the extraction methods tested. For solvent extraction experiments, an approximately 1% increase in vetiver oil yield was seen for an 8 hour extraction run (refer to Table 4.4), this is effectively a 63% improvement in yield. For hydro distillation experiments, an approximately 1.2% increase in vetiver oil yield was seen for an 8 hour extraction (refer to Table 4.5), this is approximately 6 times the yield of the 4 year old vetiver grass. Finally for SCE an approximately 0.3% increase in vetiver oil yield was seen for a 1 hour extraction at 80 bar and 40°C (refer to Table 4.6), this is effectively a 12% improvement in yield. These are significant increases when looking at such small yields however the younger vetiver plants yield a far smaller dry root mass per hectare (refer to section 5.7).

5.6. Composition Analysis

Initial observations of the vetiver oil extracts by odour and appearance indicated that for solvent extraction, hexane residues and waxy components are present in the extract, as observed in literature (refer to section 2.2.7). Hydro distillation and SCE techniques yielded a clearer extract with no hexane residues.

The experimentally obtained vetiver oil was darker in colour when compared to the standard vetiver oils from India and Indonesia (refer to Figure 4.5). This could be due to the fact that the experimentally obtained vetiver oil had not undergone any refining, which is often necessary to obtain a more valuable essential oil.

All vetiver oil extracts obtained experimentally for this research had a sweet roseate odour similar to the vetiver oil standard originating from India. The second vetiver oil standard used in this research originated from Indonesia and it had more of an earthy woody balsamic odour. Both of these odours were described in literature as the expected odour of vetiver oil (refer to section 2.1.4).

The GC-MS library available in the laboratory at the University of KwaZulu-Natal Chemical Engineering Department is the NIST Mass Spectral Database. This library contained only one of the main components known by literature (refer to section 2.1.4) to be present in the vetiver oil: zizanoic acid and only a few of the less major components. Due to the large cost in purchasing a new library specific to the flavour and fragrance industries, it was decided not to use the GC-MS and rather focus on using the GC-FID with the retention indices method to identify the unknown components (refer to section 2.3.1).

Initially two GC methods were tested: one based on Martinez et al. (2004) and the other on Danh et al. (2009) (refer to Table 2.6). These were tested by setting the GC operating parameters according to those used in each paper and analysing a sample of the experimentally obtained oil using both of the methods. In order to analyse the results by retention indices the alkane standard mixture (C6-C30) was injected for both methods.

The method proposed by Martinez et al. (2004) had no initial hold time and used larger temperature increments when compared to the method proposed by Danh et al. (2009). This resulted in the unknowns eluting from the column at a lower retention time hence lowering the total run time for analysis. The method proposed by Martinez et al. (2004) also resulted in less small traces of unknowns and the larger traces were more spread out and recognisable

In order to identify the unknown components by comparison of retention indices the same stationery phase needs to be used within the column (refer to section 2.3.1). The column stationary phase used in the research by Martinez et al. (2004) was 100% Dimethylpolysiloxane, which is consistent with the

ZB-1HT column used in this research. Therefore due to all the points above, the method and retention indices used for composition analysis was based on the publication by Martinez et al. (2004).

All experimentally obtained samples as well as two standard vetiver oils originating from India and Indonesia were analysed using the procedure described in section 3.7. Each sample of vetiver extract was analysed three times; the retention indices and peak areas for each unknown was then averaged, this eliminated uncertainties within the analysis.

From the analysis, retention times were obtained for each unknown and this retention time was converted into a retention index. To convert from retention time to index the retention times and indices of the alkane standards (refer to Figure D.1) were used in equation 2.1.

The peaks obtained from the analysis of the C6 to C30 alkane standard mixture were matched to their corresponding alkanes by assuming that the alkanes C6 to C30 elute consecutively. This was also verified by injecting pure n-octane, n-dodecane and n-hexadecane and observing that the retention times were similar to the assumed retention times within the alkane standard mixture.

Once the retention indices were calculated for all unknown components in the vetiver extract, the retention indices of the unknowns were compared to literature to identify the components. Two literature sources where found for retention indices, one from Martinez et al. (2004) and the other from the Massfinder Terpenoids Library (Hochmuth, 2011). In order to verify that the unknowns were being matched correctly with the components and indices in literature, three known samples were injected and the retention indices were calculated using the retention times and indices of the alkane standard in equation 2.1.

The retention index obtained for the known material was then compared to the retention index from the terpenoids library to verify the error (refer to Figure G.1). The terpenoids library was used for identification as this library contained components that were available in the laboratory at the University of KwaZulu-Natal Chemical Engineering Department. These components were 1-hexanol, 1-octanol and 1-decanol and they showed an error in retention indices between experimental and literature of approximately 10. This error was acceptable and hence the terpernoids library was used to identify as many of the components as possible by comparison of retention indices (refer to section 3.7). The first comparison to be discussed is the composition of vetiver oil from the two standards purchased compared to the composition of vetiver oil obtained experimentally. The vetiver oil standards from Indonesia and India were both extracted from the *Vetiveria zizanioides* species of vetiver grass and hence are comparable to the vetiver oils extracted in this study. The method of extraction used to obtain the standards was steam distillation and hence from this research the closest extraction method was hydro distillation hence the experimental results stated in Table 4.8 are from hydro distilled vetiver roots.

The vetiver oil obtained experimentally in this research by hydro distillation for a 24 hr extraction time contained only sesquiterpene alcohol derivatives with one sesquiterpene hydrocarbon. The main component was nootkatone; contributing approximately 23% to the total area of unknowns. Whereas the vetiver oil from India has an equal portion of sesquiterpene hydrocarbons and sesquiterpene alcohol derivatives with the main component being elemol which is a medium boiling alcohol.

The components in the vetiver oil sample from Indonesia were evenly distributed over a wide range of sesquiterpene hydrocarbons and sesquiterpene alcohol derivatives but is similar to the vetiver oil obtained in this research in that it to contains a substantial amount of nootkatone (8.77%). As mentioned above the vetiver oil from Indonesia has a woodier odour when compared to the vetiver oil from South Africa and India which has a sweeter odour, this has to be due to one or even a few of the sesquiterpene hydrocarbons that are not present in the composition of the other oils. To determine which component this is, one would need further investigation into the odours and properties of each component.

The specific gravity of the experimentally obtained vetiver oil was determined to be 1.515., which is very close to the specific gravity of both the standards (refer to Table 4.7). The specific gravity of the Indonesian vetiver oil 1.516 and the Indian vetiver oil 1.508.

Overall the South African vetiver oil appears to lack a variety of sesquiterpene hydrocarbons each which contribute to the odour of the vetiver oil; however it does have many sesquiterpene alcohols that also have their individual odours and contribute largely to the value of the essential oil due to their low evaporation rate which makes the oil attractive to the perfume industry. It is however assumed that the purchased standards have undergone some refining to remove unwanted components and even convert less valuable components to more valuable components.

Table 4.9 shows the comparison between the different composition of vetiver extracts when using different extractions methods and for each of these extractions method using older and younger roots.

As discussed above the vetiver oil obtained by hydro distillation for a 24 hr extraction time contained mainly sesquiterpene alcohol derivatives with only one sesquiterpene hydrocarbon and the main component was nootkatone; contributing approximately 23% to the total area of unknowns. The vetiver extract resulting from an 8 hr solvent extraction (refer to Table 4.9) had less identifiable components. It has only sesquiterpene alcohol derivatives with α-vetivone and a large amount of zizanoic acid (approximately 39%). Zizanoic acid is undesirable in vetiver oil as it precludes its use in the perfume, aromatherapy and flavour industries, however this undesirable zizanoic acid can be converted into valuable alcohols such as khusimol (refer to section 2. 4).

The main identifiable components in the extracts obtained from the SCE experiments are similar to those seen in the hydro distillation experiments. SCE extracts contain a large portion of nootkatone (approximately 10%) and a small portion of zizanoic acid (so small it can be easily eliminated). The SCE extract also contain a component that is unidentifiable (approximately 68%) , this component has been classified "unidentifiable" because it has a retention time of approximately 57 minutes which results in a higher retention index than what is found in literature from other vetiver oil samples.

Extracts obtained by hydro distillation are in a slightly lower boiling range than the solvent extraction extracts and finally the SCE extracts are in an even higher range than both hence none of the lower boiling components are produced when using SCE extraction.

It was observed that the percentage of nootkatone decreased by 12% when comparing hydro distilled extracts of 2 year old roots. However the percentage of khusimol and other sesquiterpene alcohols are increased with the appearance of a small amount of α-vetivone, which is a main contributor to odour in vetiver oil (refer to section 2.1.4).

Hydro distillation of the younger roots (2 years old), gave more identifiable components within the extract with more valuable components such as khusimol and α-vetivone. The presence of khusimol in the vetiver extract is known to be valuable (Martinez et al., 2004) due to its high presence in the standards as well as in literature (refer to and Appendix B2). The younger roots extracted by hydro distillation were only exposed to 8 hrs of extraction and hence by increasing the extraction time it is expected that there will be higher yields of extract as well as more valuable components within the extract.

The vetiver extract obtained from the younger roots by solvent extraction also by an 8 hr extraction contains a higher percentage of valuable khusimol and a lower percentage of zizanoic acid, therefore making the extract from the younger roots more valuable.

The SCE extracts extracted from the 2 yr old roots give a slightly higher percentage of nootkatone and khusimol than the 4 yr old roots and the area of the unidentifiable component is decreased to 28% for 2 year roots.

Overall the oil extracts obtained from the South African roots (Newlands Mashu) contains only a few identifiable components as compared to literature and standards (refer to Table 4.8 and Appendix B2). Majority of the identified components are sesquiterpene alcohol derivatives with α-amorphene and β-vetivenene being the only identified sesquiterpene hydrocarbon and even these are only present when hydro distillation is the chosen extraction method.

The main distinguishing factor for the extract obtained in this research is the large percentage of nootkatone when using the hydro distillation technique and the large percentage of zizanoic acid when

using the solvent extraction technique which leads one to conclude that this particular vetiver extract would be better suited in the pesticide industry as nootkatone is considered valuable in this industry (refer to section 2. 4). However the vetiver extracts did contain large percentages of khusimol which is considered valuable in the perfume industry (refer to section 2. 4).

Since the solvent extraction technique gives a fairly high yield of vetiver oil with high percentage invaluable zizanoic acid and the hydro distillation gives very low yields but no zizanoic acid with high percentages of valuable nootkatone and khusimol it is noted the SCE would be the best extraction method for these particular vetiver roots. SCE gives slightly higher yields of vetiver oil and it contains minimal zizanoic acid with higher percentages of nootkatone and khusimol. The only concern with SCE extraction is the high percentage of an unidentified component (further research into what this component could be needs to be done).

5.7. Project Feasibility

Using the vetiver oil yields obtained experimentally in this research from root material obtained at the Mashu Newlands site (South Africa), a vetiver oil yield per hectare was calculated.

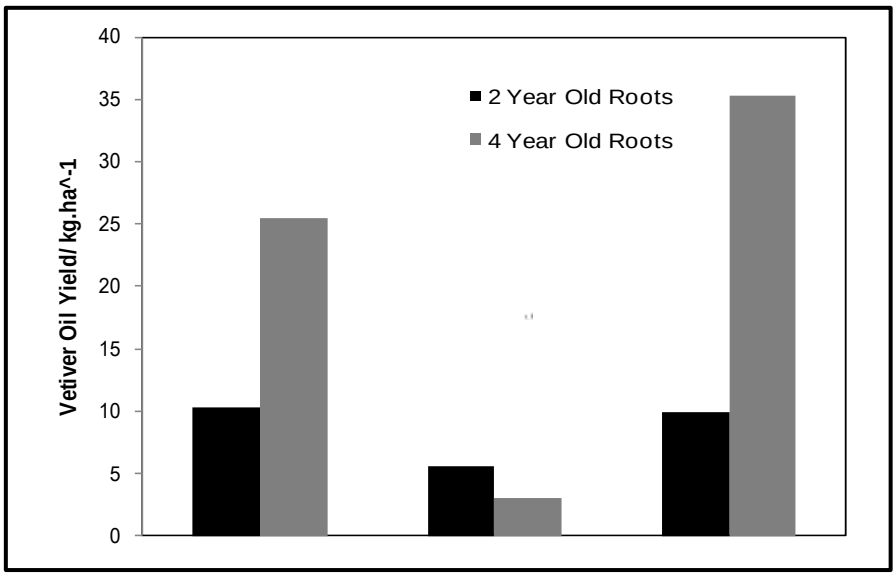


Figure 5.1. Yield of vetiver oil extracted per area of plantation.

As discussed in section 5.1 the dry root mass obtained for 2 years old roots was only 384 kg whereas the dry root mass for 4 years old roots was 1536 kg (refer to Figure 4.2). From 2 year old roots one can produce 10.4 kg vetiver oil per hectare and 4 year old roots can produce 25.5 kg vetiver oil per hectare by using solvent extraction (refer to Figure 5.1). By hydro distillation one can produce 5.5 kg vetiver oil per hectare from 2 year old roots and 3 kg vetiver oil per hectare from 4 year old roots.

Finally for supercritical CO_2 extraction one can produce 9.9 kg vetiver oil per hectare from 2 year old roots and 35.2 kg vetiver oil per hectare from 4 year old roots.

An estimate of the capital investment for pilot plant extraction setups were as follows: batch distillation pilot plant of a 50 L capacity, R 2 470 859 and supercritical carbon dioxide extractor of 24 L capacity, R 4 235 718 (refer to Table A. 3).

A preliminary feasibility study was undertaken for the batch distillation pilot plant and the supercritical carbon dioxide extractor. According to literature the typical selling prices of vetiver oil can range between R 2 000 and R 20 000 (refer to section 2.1.5) depending on quality of the oil; however since the value of the oil and hence exact selling price is unknown the worst case scenario of R 2 000 was considered in this feasibility discussion. Using this selling price, the restriction of the extractor capacity and the yields for extraction obtained in this research the total annual sales was estimated as R 17 820 and R 453 420 for the batch distillation pilot plant and the supercritical carbon dioxide extractor respectively (refer to Table A. 5). Due to the low vetiver roots produced per hectare a pilot plant facility should be sufficient for extraction however in order to increase sales one would need to increase the dry roots mass per hectare or plant more hectares of vetiver which would in turn lead to needing a larger extraction facility.

The total operating costs per annum were estimated to be R 1 223 371 and R 5 128 054 for the batch distillation pilot plant and the supercritical carbon dioxide extractor respectively (refer to Table A. 4). These operating costs are high when compared to the annual sale however this was expected due to the fact that the harvesting and cleaning of the vetiver roots is very laborious and hence operational cost would be substantial.

For the supercritical carbon dioxide extractor a big contributor to the operating costs is the cost of the carbon dioxide (CO_2) (R 3 467 870), this cost could be reduced by using a CO_2 recycle. The process of CO_2 recycling and recapture is known to have a very high capital investment and is not economically viable for small plants with small CO_2 emissions due the fact that it is a fairly complex process. However more investigations need to be done into the option of CO_2 recycling and recapture.

Due to the fact that steam distillation was not studied in detail in this study the optimum steam flowrate was not determined hence the steam costs were assumed from the study done by NEDFi ,2005 (refer to Table A.2). Additional costs for steam will be accounted for under the utility costs.

The figures stated in this preliminary feasibility study are overestimated due to the fact that scaling factors from Peters and Timmerhaus (1991) were used which are meant for full scale plant design.

From this preliminary feasibility study it is seen that the total operating costs far exceed the total annual sales and hence the business is not profitable. Therefore the production of vetiver oil from

vetiver roots is not feasible according to this research due to the high operating costs and low yields obtained from the vetiver roots. However further research needs to be done to increase the dry root mass obtained from the vetiver plantation as well as a more in depth feasibility analyses.

CHAPTER 6: CONCLUSION

Vetiver roots harvested from the Mashu Newlands Municipality site in Durban, yielded a dry root mass of 384 kg per hectare of vetiver cultivation for 2 year old roots and a dry root mass of 1 536 kg per hectare of vetiver cultivation for 4 year old roots. These dry root masses are concluded to be very low when compared to the 3000 kg of dry roots per hectare of vetiver cultivation (1.5 years old) given in literature.

The experimental apparati for solvent extraction, hydro distillation and supercritical carbon dioxide extraction were setup and tested before extraction could take place.

The yields of vetiver oil obtained from the vetiver grass harvested from Mashu Newlands plant in South Africa and extracted using the apparati setup in the laboratory were as follows; solvent extraction gave an average yield of approximately 1.66% for an 8 hour extraction of 12.5 g of dry vetiver roots using hexane as the solvent in a Soxhlet apparatus. Hydro distillation produced a yield of approximately 0.2% for an 8 hour extraction of 12.5 g of dry vetiver roots in a Clevenger apparatus and a yield of approximately 2.3% was achieved by supercritical carbon dioxide extraction (SCE) of 10.5 g of dry vetiver roots at 40˚C and 80 bar.

The vetiver oil extracts contain a large percentage of nootkatone when using the hydro distillation technique and a large percentage of zizanoic acid when using the solvent extraction technique. A minimal percentage zizanoic acid with higher percentages of nootkatone and khusimol are present in the SCE extracts. It is necessary for the vetiver oils extracted to undergo further separation and purification into more valuable oils before they can be used in the perfume and aromatherapy industry. The high present of Nootkatone indicates possible uses in the insecticide industry.

The optimum extraction method in terms of high yield and valuable components would be supercritical carbon dioxide extraction (SCE). For a pilot scale SCE extractor the total annual sales was estimated as R 453 420 and the total operating costs per annum were estimated to be R 4 839 813. Therefore from this preliminary feasibility study it is seen that the total operating costs far exceed the total annual sales and hence the business is not profitable.

CHAPTER 7: RECOMMENDATIONS

Due to the effects of growing conditions on vetiver oil production it is recommended that research into the agricultural aspects of the project be done; specifically into the optimum growing conditions to maximise the dry root mass which at the moment is very low.

Optimization of the supercritical carbon dioxide extraction equipment is also recommended for more convenient operation and to increase the vetiver oil yield obtainable from the equipment. The micro metering valve at the exit of the system requires better control. To increase the yield of vetiver oil it is recommended to add an ethanol co-solvent to the system.

The root mass yield at the present site may be low but further studies could be undertaken on root mass obtained from pontoons and other sources (if the objective is to set up some small enterprises for the community development). As well as a more in depth feasibility study.

The final recommendation is that a more in depth analysis into the composition of the vetiver oil using an applicable GC-MS library is undertaken. Another method for analysis would be to purchase standards of the various components of vetiver oil to form a reference for identification however availability was a challenge when it came to these components.

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APPENDIX A

A.1. Sample Calculation for conversion of prices

- For R 93/ 11ml:

Specific Gravity of vetiver oil from India = 0.9958 (refer to Table B.9)

Conversion from a volume to a mass basis:

$$11\text{mL} \times \frac{1\text{L}}{1000\text{mL}} \times \frac{1\text{m}^3}{1000\text{L}} \times \frac{995.8\text{kg}}{\text{m}^3} = 0.011\text{kg}$$

Therefore the cost per kg is R8 454.5.

- For US $6.95/ 2.5ml:

Specific Gravity of vetiver oil from Haiti = 1.01 (refer to Table B.9)

US $1 = R7 Average of latest trends

Conversion from a volume to a mass basis:

$$2.5\text{mL} \times \frac{1\text{L}}{1000\text{mL}} \times \frac{1\text{m}^3}{1000\text{L}} \times \frac{1010\text{kg}}{\text{m}^3} = 0.0025\text{kg}$$

Conversion from US $ to R:

$$\$6.95 \times \frac{\text{R7}}{\$} = \text{R48.65}$$

$$\therefore \text{R48.65}/0.0025\text{kg}$$

Therefore the cost per kg is R19 460.

- For US $ 13.5/1oz:

Conversion from oz to kg: 1kg = 35.3oz

Conversion from US $ to R:

$$\$13.50 \times \frac{\text{R7}}{\$} = \text{R94.50}$$

Therefore the cost per kg is R3 335.85

A.2. Typical Selling Prices of Vetiver Oil

Table A. 1. Typical selling Prices

Selling Price*	(R/kg)	Supplier	Source
R93 for 11ml	8 455	Esoteric Oils - India	www.essentialoils.co.za/essential-oils/vetiver.htm [18/01/11]
US $6.95 for 2.5ml	19 460	Health Mastery Systems - Haiti	www.kgstiles.com/vetivermoreinfo.htm [18/01/11]
US $276.29/kg	1 934	Ghangsha Guanxiang Chemicals Trading Co. Ltd. - China	www.aliexpress.com/product-gs/358765513-Wholesale-1kg-Pure-Natural-Vetiver-Essential-Oil-Vetiver-Oil-wholesalers.html [18/01/11]
US $355.55/kg	2 489	Lala Jagdish Prasad & Company - India	www.naturalfragrances.net/vetiver-oil.html [18/01/11]
US $13.5 for 1oz	3 336	100% Pure Essential Oils - Indonesia	http://100pureessentialoils.com/categories/Pure-Essential-Oils/Vetiver-Oil/ [18/01/11]

*R1546/kg as used in the above Calculation (NEDFi, 2005)
*Refer to appendix A1 for conversions

A.3. Economics of Vetiver Cultivation

The following economic evaluation was done per hectare of vetiver grass cultivation by the NEDFi (2005):

Expected yield: Cost of cultivation and processing per hectare basis (18 months duration)

Yield of air-dry root 3 000 kg/ha

Yield of oil at about 0.4 % recovery 12 kg/ha

Table A. 2. Cost Summary (NEDFi, 2005)

Operation	Cost (R)*
Cost of seedlings	3 093
Field preparation and bed formation	464
Planting & gap filling	464
Manures & fertilizers application	619
Intercultural-weeding, hoeing & earthing up	387
Top removing –twice	155
Harvesting of roots	696
Cost of cleaning / shade drying of roots/ packing/ carrying	108
Distillation cost @ R 124/kg oil assuming 12 kg per hectare	1 485
Filtering & packing of oil	108
Miscellaneous expenditures	155
Total expenditure	7 732
Gross return @ R 1 546/ kg x 12	(18 557)
Net return	10 825
When only dry root is produced and sold:	
Cost up to dry root production	5 985
Gross return @ R 4/ kg of root	11 598
Net return	5 613

* 1 Indian Rupee (Rs) = 0.15464 South African Rand [15/02/11]: Original source in Rs.

A.4. Preliminary Estimation of Costs for an Extraction Plant

Table A. 3. Capital investment estimations for a pilot plant extractor.

Type of Pilot Plant:	**Batch distillation pilot plant**	**Supercritical fluid extraction plant**
Extraction techniques	Steam &/ Hydro distillation	Supercritical Fluid extraction
	Built from parts	1 unit
Capacity of extractor /L	50	24
Material	Glass	Stainless Steel
Total Equipment Cost *: With Safety factor	R 397 207	R 873 810
Purchased equipment installation	R 154 911	R 340 786
Instrumentation and controls (installed)	R 51 637	R 113 595
Electrical (installed)	R 39 721	R 87 381
Building (including services)	R 115 190	R 253 405
Yard Improvement	R 39 721	R 87 381
Service facilities (installed)	R 218 464	R 480 596
Engineering and supervision	R 127 106	R 279 619
Construction expenses	R 135 050	R 297 095
Total indirect and direct costs:	R 1 279 008	R 2 813 668
Contractor's fee	R 63 950	R 140 683
Contingency	R 127 901	R 281 367
Chemical Analysis	**Gas Chromatograph**	
GCMS Cost: * Shimadzu 2010, FID, Capillary column	R 1 000 000	
Total Capital Investment	R 2 470 859	R 4 235 718

REF: Peters and Timmerhaus (1991)

* Total equipment costs from suppliers Wenzhou Chengdong Import and Export Co., Ltd and QVF: De Dietrich Process Systems for the supercritical fluid extraction and steam distillation plants respectively (Prices valid: June 2011).

Table A. 4. Estimation of annual total operating costs

Operating Costs		
	Steam Distillation	**Supercritical CO_2 Extraction**
Fixed Costs:		
Insurance	R 25 133.60	R 43 292.16
Salaries (Admin)**	R 180 000.00	R 180 000.00
Research and Development	R 356.40	R 9 068.40
Total fixed costs*	R 205 490.00	R 232 360.56
Variable Costs		
Raw Materials:	Steam ***	Carbon Dioxide****
Extraction fluid	R 1 104.84	R 3 467 870.36
Vetiver Seedlings(NEDFi, 2005)	R 36 868.56	R 70 829.70
Fertiliser (NEDFi, 2005)	R 7 378.48	R 14 175.10
Operating Labour**	R 600 000.00	R 600 000.00
Operating Supervision**	R 240 000.00	R 240 000.00
Utilities	R 111 215.59	R 466 186.76
Maintenance and Repairs	R 7 944.15	R17 476.20
Operating Supplies	R 1 191.62	R 2 621.43
Laboratory Charges	R 6 000.00	R 6 000.00
Safety and Protection	R 6 000.00	R 6 000.00
Transportation Cost	R 178.20	R 4 534.20
Total variable costs*	R 1 017 881.43	R 4 895 693.75
Total operating costs*	R 1 223 371.43	R 5 128 054.31

REF: Peters and Timmerhaus (1991)

Assumptions:
* 330 working days a year
**Labour - 1 administrative / financial / human Resources @ R 15 000 per month / R 10 000 per operator per month 1 per shift 3 shifts / R 5 000 per harvester per month 4 people 1 shift / 1 supervisor @ 20000 per month
***Steam cost = Distillation cost (NEDFi, 2005) (refer to Table A.2)
**** Assume constant superficial velocity inside the extraction bed during scale up of CO2 flowrate / R450 per 31.5kg Airflex/ extractor bed diameter of 20 cm for pilot plant

Table A. 5. Estimation of annual sales

	Sales	
Production rate/ kg.day^{-1}**	0.027	0.687
Selling Price(R.Kg^{-1})	2000	2000
Total Sales *	R 17 820.00	R 453 420.00

Assumptions:
* 330 working days a year
**Based on 92.5 kg.m^{-3} bed density, 24 L extraction capacity, 2 hr runs for 24 hrs a day and 2.58% oil yield (dry root basis) for the supercritical CO_2 extraction.
Based on 92.5 kg/m3 bed density, 50 L extraction capacity, 8hr runs for 24 hrs a day and 0.198% oil yield (dry root basis) for the steam distillation.

APPENDIX B

B.1. Tabulated yields of vetiver oils from vetiver grass found in literature

Table B. 1. Yields and extraction times for the extraction of vetiver oil from Brazilian vetiver roots using different extraction methods (Martinez et al, 2004).

Method	Extraction time/ h	Yield/ %
Hydro distillation (HD)	16	1.8 +/- 0.1
HD- Nitrogen milling	16	1.8 +/- 0.1
HD-Sodium hydroxide	16	1.8 +/- 0.1
HD-Enzymes	16	1.9 +/- 0.1
HD-Sodium hydroxide and enzymes	16	1.7 +/- 0.1
SFE (200bar, 40°C)	1	3.2 +/- 0.2
HD from SFE extract	12	0.6 +/- 0.1

Table B. 2. Yields and operating parameters for the extraction of vetiver oil from vetiver roots using different extraction methods (Danh et al., 2009).

Method	Operating Conditions				Yields /%
	Temperature /°C	Time /min		Pressure /bar	
		Static	Dynamic		
HD	100	-	720	-	0.31 +/- 0.01
SE	70	-	300	-	1.91+/- 0.19
SCE	50	30	100	190	1.38

*HD – hydro distillation; SCE – supercritical carbon dioxide extraction; SE – solvent extraction

Table B. 3. Yields for the extraction of vetiver oil from vetiver roots using SCE at varying operating parameters (Danh et al., 2009).

Experiment	Pressure /bar	Temperature /°C	Time /min	Mass of CO_2 used /g	Yield /%
1	100	40	50	95	0.6
2	190	40	50	100	1.11
3	100	50	50	95	0.36
4	190	50	50	100	1.19
5	100	40	100	190	0.72
6	190	40	100	200	0.95
7	100	50	100	190	0.46
8	190	50	100	200	1.38
9	69.3	45	75	140	0.13
10	220.7	45	75	152	0.78
11	145	36.6	75	147	0.84
12	145	53.4	75	147	1.03
13	145	45	33	65	0.8
14	145	45	117	229	0.83
15	145	45	75	147	0.84
16	145	45	75	147	0.8
17	145	45	75	147	0.77
18	145	45	75	147	0.74
19	145	45	75	147	0.82

Table B. 4. Yields and operating parameters for the extraction of vetiver oil from vetiver roots using different extraction methods (Danh et al., 2010).

Method*	Operating Conditions				Yields /%
	Temperature /°C	Dynamic Time /min	Pressure /bar	Ethanol (vol. %)	
HD	100	720	-	-	1.69 +/- 0.07
SCE	50	105	190	-	3.74+/- 0.12
mod. SCE	50	105	190	15	5.90

*HD – hydro distillation; SCE – supercritical carbon dioxide extraction; mod. SCE – ethanol modified supercritical carbon dioxide extraction

Table B. 5. Yields for the extraction of vetiver oil from vetiver roots using SCE at varying operating parameters (Danh et al., 2010).

Experiment	Pressure /bar	Temperature /°C	Ethanol (vol. %)	Yield /%
1	100	40	5	3.58
2	190	40	5	4.78
3	100	50	5	2.47
4	190	50	5	4.48
5	100	40	15	5.31
6	190	40	15	5.77
7	100	50	15	5.02
8	190	50	15	5.90
9	145	37	10	4.29
10	145	53	10	4.73
11	145	45	2	3.39
12	145	45	18	4.84
13	145	45	10	4.69
14	145	45	10	4.90
15	145	45	10	4.95
16	145	45	10	4.83
17	145	45	10	5.02

B.2. Composition analysis results of vetiver oil found in literature

Table B. 6. Chemical Composition of the Volatile Fractions of Vetiver Extracts (Percentage of Total) (Martinez et al., 2004)

Compound	SFE Extract /% Area	Hydro-distilled Extract /% Area	Retention Index 1*	Retention Index 2**
α-ylangene		0.1	1465	
Pre-zizaene		0.6	1590	1375
Khusimene	0.5	0.5	1620	1468
α-amorphene	0.3	0.4	1676	1491
Cis-eudesma-6,11-diene			1692	1498
Cis-β-guaiene	0.2	0.8	1702	
δ-amorphene	0.3	0.2	1710	1519
β-vetispirene		0.2	1737	1506
γ-cadinene	0.2	0.3	1752	1531
γ-vetivenene			1813	1540
β-vetivenene		0.4	1852	1574
α-calacorene			1914	1552
Cis-eudesm-6-en-11-ol	1.5	1.7	2064	1575
Khusimone	2.4	2.6	2175	1616
Ziza-6(13)-en-3-one	1.8	2.0	2227	
Khusinol	1.5	2.2	2292	1699
Khusian-2-ol	1.6	2.4	2323	1715
Vetiselinenol	0.8	1.3	2343	
Cyclocopacamphan-12-ol	0.6	0.8	2351	
2-epi-ziza-6(13)-3α-ol	1.1	1.9	2406	
Isovalencenal	1.8	1.5	2453	
β-vetivone	0.8	1.9	2519	1829
Khusimol	7.2	9.5	2521	1774
Nootkatone	1.2	1.1	2539	1819
α-vetivone	5.4	4.9	2559	1851
Isovalencenol	7.4	8.3	2567	1813
Bicyclovetivenol	1.2	0.2	2604	
Zizanoic acid	32.4	24	>2800	1837
Total Hydrocarbons	1.5	3.5		
Total Alcohols	22.9	28.3		
Total Carbonyl compounds	13.4	14		
Total Carboxylic acids	32.4	24		
Total Identified	70.2	69.8		

*RI1 - retention index on Carbowax column; **RI2 - retention index on DB1 column

Table B. 7. Chemical Composition of the Volatile Fractions of Vetiver Extracts (Percentage of Total) (Danh et al., 2010)

Compound	SFE Extract* /% Area	Hydro-distilled Extract*/% Area	Kovats Index
Acoradiene		0.19	1403
β-copaene		0.31	1426
Prezizaene	0.46	0.79	1438
Khusimene	0.6	1.05	1443
Calarene	0.17	0.38	1451
Trans-isolimonene		0.32	1459
α-Amorphene		0.89	1478
β-vetispirene		0.56	1488
δ-selinene		0.8	1490
γ-Amorphene		0.26	1494
Cuparene		0.28	1507
δ-Amorphene		0.21	1511
Nootkatene			1512
α-cadinene	0.4	0.76	1519
α-calacorene		0.26	1544
β-Vetivenene		1.49	1552
Virodoflorol	0.53	0.57	1595
Khusimone	0.68	1.38	1601
Epi-α-Cadinol	0.51		1648
Pogostol	0.43	0.85	1651
7-epi-α-Eudesmol	0.77	1.07	1661
Epi-zizanone	2	2.5	1671
Epi-nootkatol	1.21	1.9	1683
Khusinol			1687
Zizanal	1.18	1.72	1700
Juniper camphor	0.98		1722
Vetiselinenol	2.26	3.65	1727
Khusimol	11.63	14.3	1745
14-Hydroxy-δ-Cadinene		0.42	1769
Isovalencenol	5.65	7.26	1792
Nootkatone		5.71	1809
Zizanoic acid	15.16	0.68	1817
β-Vetivone	2.35	2.62	1820
Sesquiterne Ketone	3	3.35	1830
α-vetivone	6.4	7.33	1843
Hexadecanoic acid	0.97	0.25	1971
Total Hydrocarbons	1.63	8.97	
Total Alcohols	23.97	29.6	
Total Carbonyl compounds	15.61	24.6	
Total Carboxylic acids	16.12	0.93	
Total Identified	57.33	64.11	

***** Deviations have not been stated in the table.

Table B. 8. Chemical Composition of the Volatile Fractions of Vetiver Extracts (Percentage of Total) (Danh et al., 2009)

Compound	SFE Extract* /% Area	Hydro-distilled Extract* /%Area	Hexane Extract* /% Area	Kovats Index
α- Ylangene				1363
α- Duprezianene				1372
β- Funebrene		0.12		1405
β- Copaene		0.21		1429
Prezizaene		0.14		1441
Khusimene		0.17		1446
α-Patchoulene				1454
α- Amorphene		0.31		1481
β- Vetispirene				1491
δ- Selinene				1493
γ- Amorphen				1497
Cuparene				1507
δ-Amorphene				1511
Nootkatene				1515
γ-Cadinene	0.94			1519
Valencene				1522
δ-Cadinene				1529
γ-Vetivenene				1534
10-epi-cis-Dracunculifoliol				1538
α-Calacorene		0.11		1547
Elemol				1551
β-Vetivenene		0.28		1556
Sphathulenol				1589
Viridoflorol	0.55			1596
Khusimone	0.98	1.34	0.85	1604
Epi-α-Cadinol	0.12	0.71	0.34	1646
Pogostol	0.15	0.48	0.21	1651
Valerianol		0.95	1.87	1661
Epi-zizanone	1.09	2.23	0.97	1671
Khusinol				1687
Eudesma-4(15),7-dien-1βol	2.16		0.78	1700
Zizanal	0.86			1701
Juniper camphor		1.35		1721
Vetiselinenol	2.08	3.63	1.27	1730
Khusimol	15.54	25.8	13.3	1747
14-Hydroxy-δ-cadinene	0.78	1.03	0.7	1759
Isovalencenol	4.25	6.64	3.43	1795
Nootkatone	1.39	1.12		1812
Zizanoic acid	25.88	9.6	31.1	1818
β-Vetivone	2.48	1.97		1824
Sesquiterne ketone	3.54	2.31	1.93	1833
α-Vetivone	6.03	6.77	5.42	1845
Hexadecanoic acid	1.21	1.86	1.92	1975
Total Hydrocarbons	0.78	2.37	0.7	
Total Alcohols	25.7	39.56	21.23	
Total Carbonyl compounds	16.37	15.74	9.17	
Total Carboxylic acids	27.09	11.46	32.98	
Total Identified	69.94	69.13	64.08	

* Deviations have not been stated in the table.

B.3. Physical properties of vetiver oil

Table B. 9. Physical properties of vetiver oil from various locations

Location	India - Bharatpur*	Haiti**	Indonesia ***
Specific Gravity @ 20°C	0.9958	0.98400 - 1.03500	0.978 – 1.038
Refractive index @ 20°C	1.5147	1.52100 - 1.52600	1.513 – 1.530
Optical Rotation	-65.2”	+17.00 - +46.00	+15 – +45
Acid Value	40.6		
Ester Value	36.5		
Ester value after acetylation	146..6		
Total Alcohol as $C_{15}H_{24}O$	64.9		
Carbonyl value	20%		
Solubility at 80% alcohol	1:2		

* Chowdhury et al., 2002
** The Good Scents Company: Vetiver Oil Haiti MSDS, 2011
***New Directions Aromatics: Vetiver oil MSDS, 2010

APPENDIX C

C.1. Design of the Supercritical Carbon Dioxide Extraction Setup

Tube Sizing

From fluid density assume an optimum velocity (u /m.s^{-1}) (Simpsons):

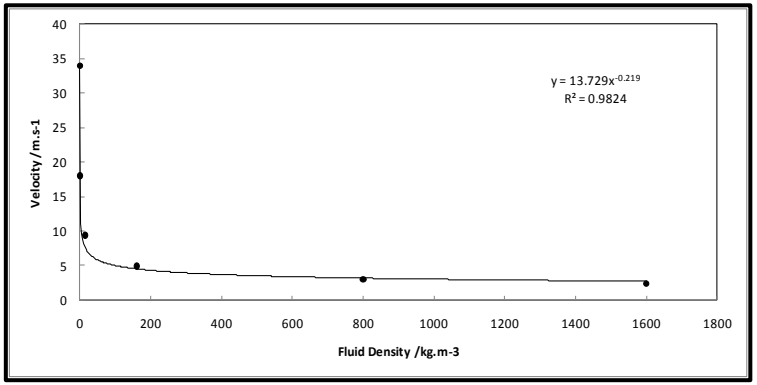


Figure C. 1. Optimum fluid velocity through a pipe. (Coulson and Richardson, 2006)

Table C. 1. Selection of tube size based on the various phases of operation.

	P /bar	T / K	ρ_f / kg.m-3	u / m.s-1	Q / m3.s-1	A /m^2	di / mm	t / mm	Standards according to specs:				
									do /mm	di /mm	t /mm	Pmax / bar	in
Critical point	73.8	304	468	3.57	1.47E-07	4.1E-08	0.229	0.020					
Supercritical High	200	313.15	830	3.15	8.31E-08	2.6E-08	0.183	0.016					
Supercritical Low	80	313.15	284	3.98	2.43E-07	6.1E-08	0.279	0.024	3.175	2.464	0.711	586	0.125
Gas	1.013	288.15	1.87	11.97	3.69E-05	3.1E-06	1.982	0.172					
Liquid	19.7	253.15	1032	3.00	6.69E-08	2.2E-08	0.168	0.015					

Pi = 22 $N.mm^{-2}$

S= 138 $N.mm^{-2}$ .....For 316 SS @ 40°C (Coulson and Richardson, 2006)

m = 6.9 x 10^{-5} $kg.s^{-1}$..... (Martinez et al., 2004)

$$\dot{Q} = \frac{\dot{m}}{\rho_f}$$

$$A = \frac{\dot{Q}}{u} = \frac{\pi d_i^2}{4}$$

$$t = \frac{P_i D_i}{2S - P_i}$$

Extraction Vessel Sizing

V = 100 ml

Di = 2 cm .....Selected based on Martinez et al., 2004 (Refer to section 3.6)

L = 31.8cm

Vessel Thickness:

$$t = \frac{P_i D_i}{2S - P_i} = \frac{22 \times 20}{2 \times 138 - 22} = 1.73\ mm$$

$t_{act} = 20$ mm..... (Refer to section 3.6)

Bolt Selection:

d_{bolt} = 6mm

De = 50mm

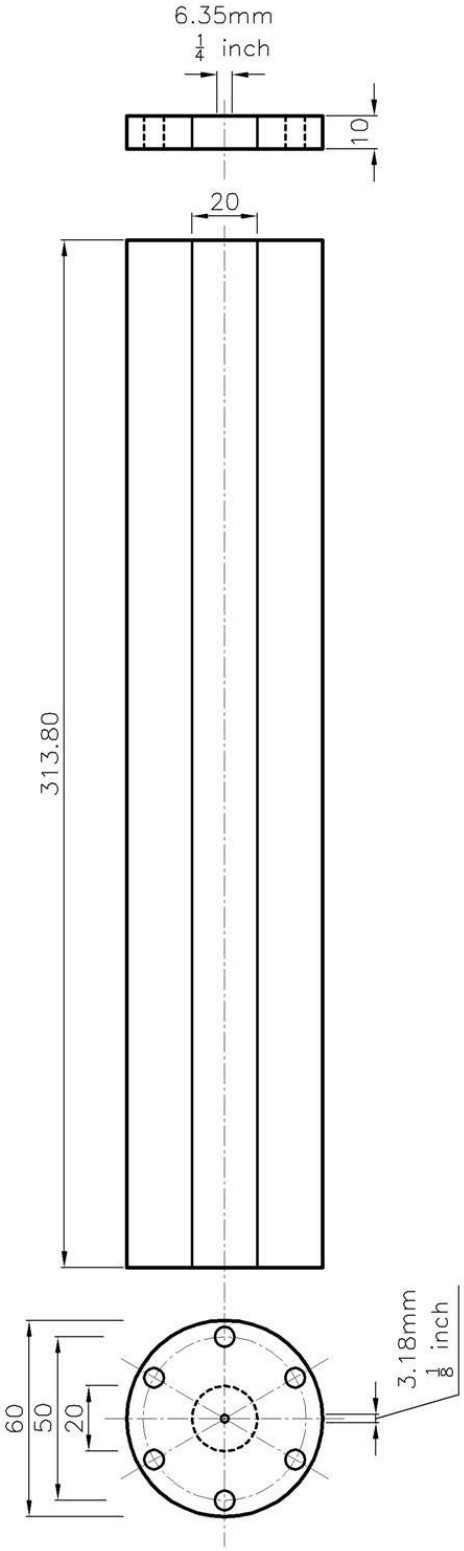
Flat Ends:

C = 0.25 .....Bolted cover with full-face gasket (Coulson and Richardson, 2006)

E = 1 .....No welding of the flat end

$$t_f = D_e \sqrt{\frac{CP_i}{SE}} = 50 \sqrt{\frac{0.25 \times 22}{138 \times 1}} = 9.98mm$$

$t_{fact} = 10\ mm$..... (Refer to section 3.6)



Extraction Cell	Equipment no: E101
Operating data	
Design flow rate /kg.s^{-1}	6.9 x 10^{-5}
Design temperature /°C	50
Design pressure /bar	220
Design data	
Vessel volume /ml	100
Vessel Inside diameter /m	0.02
Vessel height /m	0.3138
Bed density /kg.m^{-3}	92.5
Mechanical design	
Material of construction	Stainless steel - 304
Vessel thickness /mm	20
Design stress /N.mm^{-2}	138
Thickness of flat ends /mm	10
Bolt diameter /mm	6
Bolt circle diameter /mm	50

UNIVERSITY OF KWAZULU NATAL
SCHOOL OF CHEMICAL ENGINEERING

SUPERCRITICAL CARBON DIOXIDE EXTRACTION CELL
NTS

DRAWN BY: Bianca Leite
DATE: 15 July 2011

Calculation of the Heating/Cooling Coil Length

Table C. 2. Heat capacities of carbon dioxide at various temperatures and pressures (Coulson and Richardson, 2006)

	P /bar	T / K	Cp /KJ.kg^{-1}K^{-1}
Into cooling coil	80	298.15	2.805
Out of cooling coil	80	278.13	2.303
Into heating coil	200	278.13	2.010
Out of heating coil	200	313.15	2.303

* Higher Cp gives worst case due to longer length of coil.

U = 0.1 KW.m^{-}2.K^{-1} ..... (Coulson and Richardson, 2006)

Heating Coil

T_{in} = 5°C

T_{out} = 40°C

$$q = \dot{m}cp\Delta T = 6.9 \times 10^{-5} \times 2.302 \times (40 - 5) = 0.00556\ KJ.s^{-1}$$

$$q = UA\Delta T \therefore A = \frac{q}{U\Delta T} = \frac{0.00556}{0.1 \times (40 - 5)} = 0.00159\ m^2$$

$$A = \pi d_i L \ \therefore L = \frac{A}{\pi d_i} = \frac{0.00159}{\pi \times 2.4638/1000} = 0.205\ m$$

Cooling Coil

T_{in} = 25°C

T_{out} = 5°C

$$q = \dot{m}cp\Delta T = 6.9 \times 10^{-5} \times 2.805 \times (5 - 25) = -0.00387\ KJ.s^{-1}$$

$$q = UA\Delta T \therefore A = \frac{q}{U\Delta T} = \frac{-0.00387}{0.1 \times (5 - 25)} = 0.00194\ m^2$$

$$A = \pi d_i L \ \therefore L = \frac{A}{\pi d_i} = \frac{0.00194}{\pi \times 2.4638/1000} = 0.25\ m$$

Table C. 3. Hazard and Operability Study on the extraction vessel

Vessel:	Extractor E101			
Design Intent:	To hold the bed of plant material for extraction.			
Guide Word	**Process Parameter**	**Cause**	**Consequence**	**Action**
No High	Flow Pressure	Blockage	Build up of pressure therefore possible rapture	-Regularly wash the system with hexane -Relief valve RV1 (Set-200bar)
Reverse	Flow	Blockage	Pump Failure	Check Valve CV1 (Non return)
Vessel:	HPLC Pump P101			
Design Intent:	To supply pressure and control flow of the fluid within the system (Carbon Dioxide).			
Guide Word	**Process Parameter**	**Cause**	**Consequence**	**Action**
No	Flow	Pump fails	Low pressure in extractor	Regularly wash the system with hexane
High	Temperature	Cooler/ Chiller (C101) fails	Pump fails due to gas in feed	Monitor temperature
Reverse	Flow	Blockage upstream of the pump	No extraction	Check Valve CV1 (Non return)
Vessel:	Glass Trap T101			
Design Intent:	To collect the extract after extraction.			
Guide Word	**Process Parameter**	**Cause**	**Consequence**	**Action**
No	Flow	Blockage	Pressure builds up downstream	Relief valve RV1 (Set-200bar)
High	Pressure	BPR1 fails	Rupture of glass trap	Regularly wash the system with hexane

APPENDIX D

D.1. Gas chromatograph Results

* All graphs displayed below were obtained using the method shown in section 3.7.

Alkane Standard

Table D. 1. GC analysis results for the C6 - C30 alkane standard.

Peak#	Retention Time /min	Area	%Area
1	2.64	294470	0.05
2	2.73	663815	0.11
3	2.98	563131818	97.11
4	3.12	2503290	0.43
5	3.47	325013	0.06
6	4.05	782010	0.13
7	6.74	803179	0.14
8	11.03	794862	0.14
9	16.10	784889	0.14
10	21.24	818400	0.14
11	26.17	792279	0.14
12	30.83	759545	0.13
13	35.22	730414	0.13
14	39.36	683705	0.12
15	43.26	670121	0.12
16	46.98	633352	0.11
17	50.51	593233	0.10
18	53.87	542535	0.09
19	57.08	495026	0.09
20	60.15	444631	0.08
21	63.09	398372	0.07
22	65.90	363796	0.06
23	68.62	362443	0.06
24	71.61	359837	0.06
25	75.29	360195	0.06
26	79.93	342063	0.06
27	85.84	218849	0.04
28	93.46	120992.6	0.02

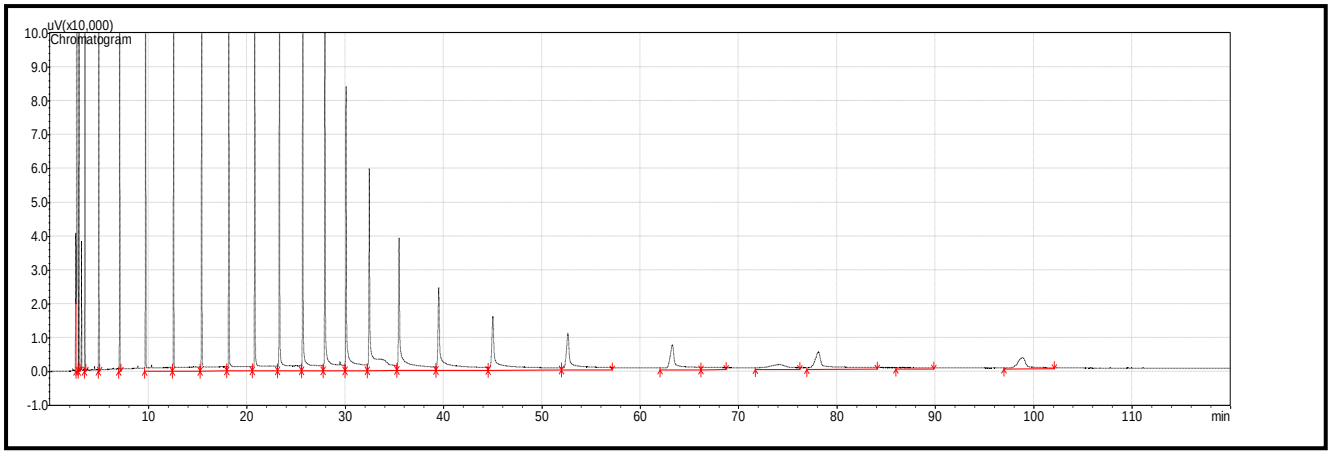


Figure D. 1. GC graph from the analysis of the C6 - C30 alkane standard.

Comparison of standards:

Table D. 2. GC analysis results for the standard vetiver oil from India.

Peak#	Retention Time /min	Area	%Area
1	2.37	153949	0.10
2	2.47	139667356	87.65
3	2.74	17177873	10.78
4	21.23	273168	0.17
5	22.39	371672	0.23
6	24.25	494772	0.31
7	24.49	235396	0.15
8	28.37	204770	0.13
9	29.31	156418	0.10
10	29.62	138852	0.09
11	30.29	208247	0.13
12	34.53	124251	0.08
13	55.10	147679	0.09

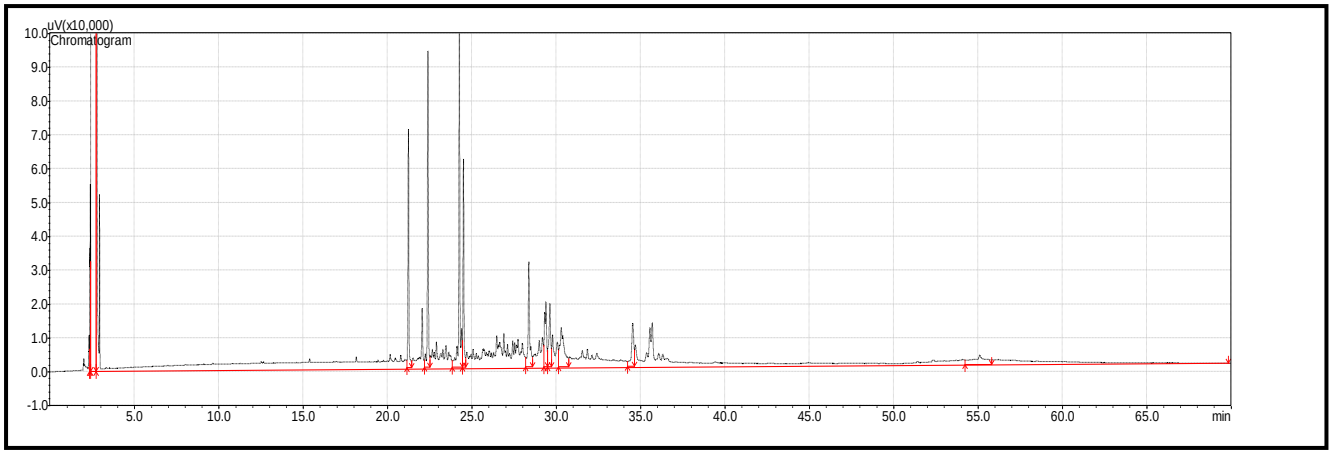


Figure D. 2. GC graph from the analysis of standard vetiver oil from India.

Table D. 3. GC analysis results for the standard vetiver oil from Indonesia.

Peak#	Retention Time /min	Area	%Area
1	2.60	168992	0.12
2	2.69	67493903	49.31
3	2.76	64533459	47.14
4	2.87	297671	0.22
5	2.92	924528	0.68
6	20.46	164186	0.12
7	21.22	117113	0.09
8	22.05	184345	0.13
9	22.64	118087	0.09
10	22.77	107585	0.08
11	22.90	190497	0.14
12	23.75	219487	0.16
13	24.38	217807	0.16
14	24.51	130366	0.10
15	25.13	148188	0.11
16	25.79	184590	0.13
17	26.48	155933	0.11
18	26.65	154245	0.11
19	26.89	106921	0.08
20	27.11	110073	0.08
21	27.77	152154	0.11
22	28.38	294831	0.22
23	29.29	104368	0.08
24	29.79	321568	0.23
25	30.29	178340	0.13
26	54.11	107407	0.08

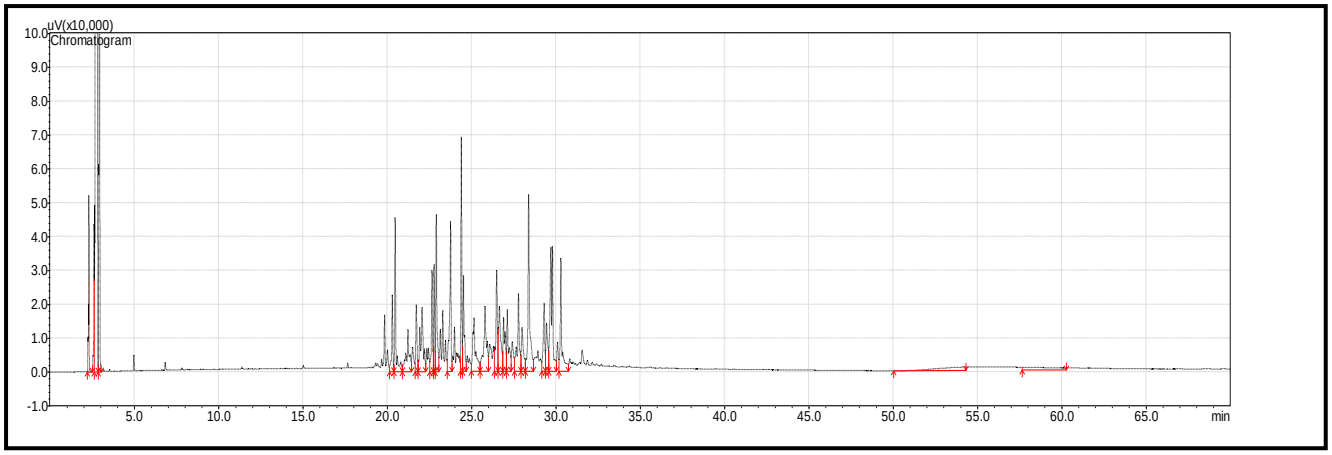


Figure D. 3. GC graph from the analysis of standard vetiver oil from Indonesia.

Comparison of Extraction Techniques:

Table D. 4. GC analysis results for the vetiver oil obtained by Hydro Distillation (8 hr extraction) – 4 Years.

Peak#	Retention Time /min	Area	%Area
1	2.60	168992	0.12
2	2.69	67493903	49.31
3	2.76	64533459	47.14
4	2.87	297671	0.22
5	2.92	924528	0.68
6	20.46	164186	0.12

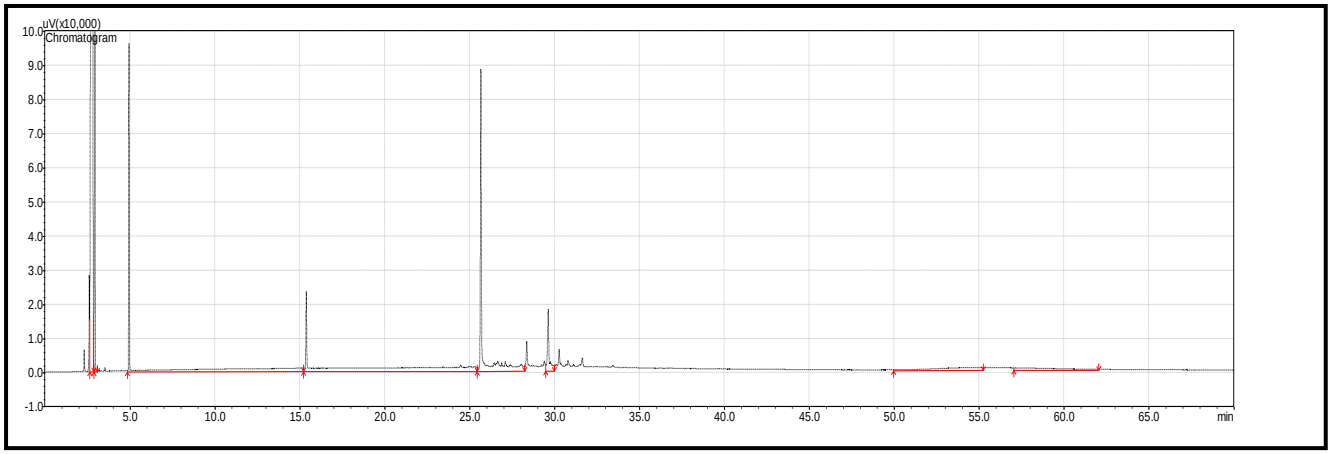


Figure D. 4. GC graph from the analysis of vetiver oil obtained by Hydro Distillation (8 hr extraction) – 4 Years.

Table D. 5. GC analysis results for the vetiver oil obtained by Solvent Extraction (8 hr Extraction) – 4 Years.

Peak#	Retention Time /min	Area	%Area
1	2.66	246056	0.19
2	2.76	120808186	94.43
3	2.93	2674926	2.09
4	28.38	303370	0.24
5	29.40	191377	0.15
6	29.89	1708270	1.34
7	30.03	102056	0.08
8	30.30	328083	0.26
9	30.52	193227	0.15
10	30.83	126813	0.10
11	31.09	108947	0.09
12	31.64	347161	0.27
13	32.25	165373	0.13
14	33.01	151691	0.12
15	33.49	104730	0.08
16	52.49	101243	0.08
17	54.79	136712	0.11
18	57.18	136689	0.11

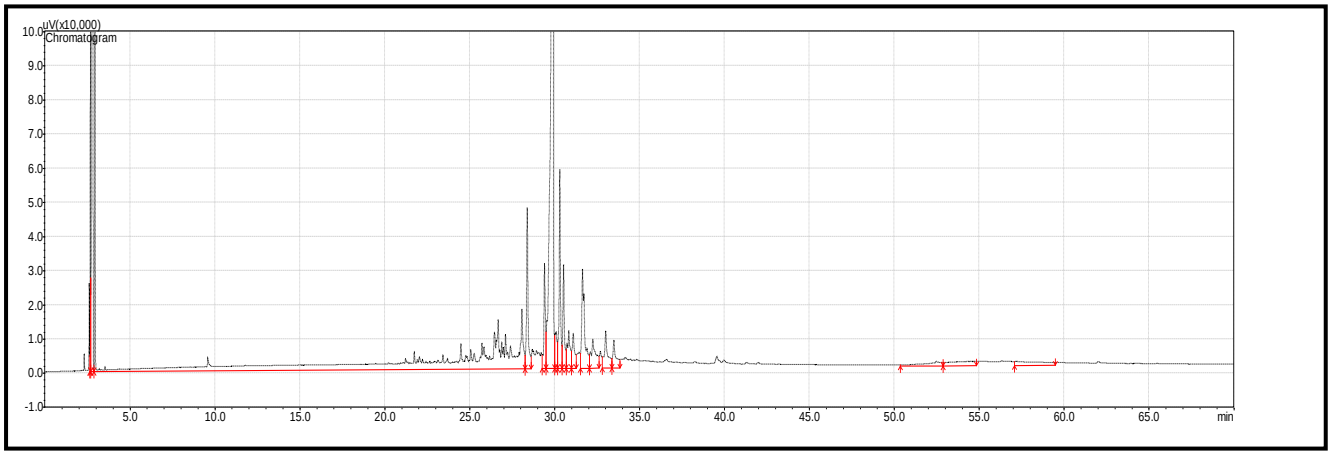


Figure D. 5. GC graph from the analysis of vetiver oil obtained by Solvent Extraction (8 hr Extraction) – 4 Years.

Table D. 6. GC analysis results for the vetiver oil obtained by Supercritical CO_2 Extraction (80 bar/40°C/1 hr) – 4 Years

Peak#	Retention Time /min	Area	%Area
1	2.63	219739	0.15
2	2.73	137680752	92.66
3	2.89	957801	0.64
4	25.70	169051	0.11
5	26.53	143582	0.10
6	27.98	131194	0.09
7	28.27	471927	0.32
8	29.27	393739	0.27
9	29.70	926663	0.62
10	29.98	148473	0.10
11	30.19	265151	0.18
12	30.38	120033	0.08
13	31.52	231058	0.16
14	57.32	6725843	4.53

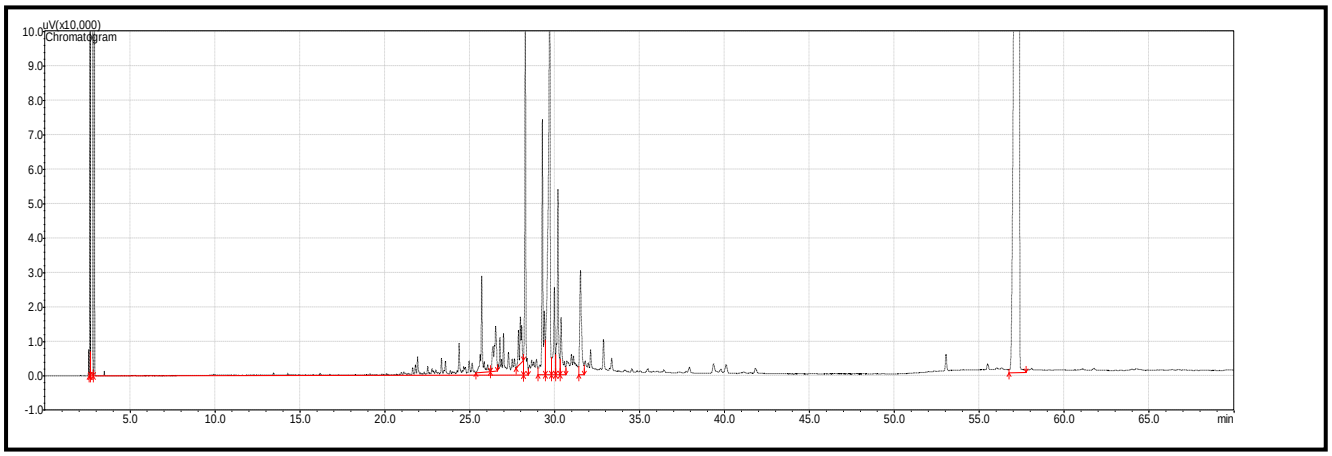


Figure D. 6. GC graph from the analysis of vetiver oil obtained by Supercritical CO_2 Extraction (80 bar/40°C/1 hr) – 4 Years

APPENDIX E

E.1. Evaporation Rate of Hexane from Vetiver Extract

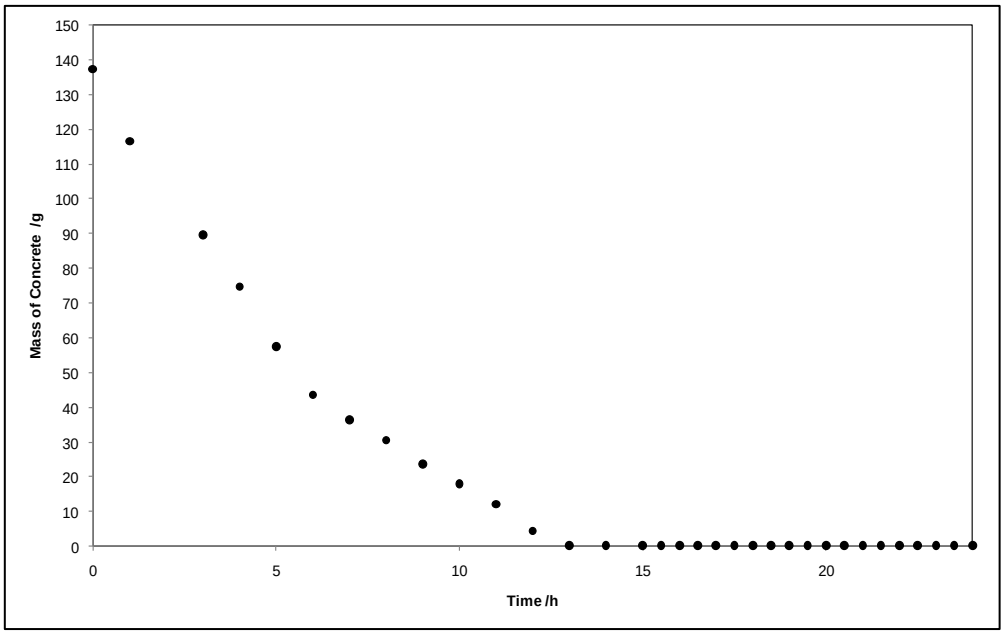


Figure E. 1. Evaporation rate of hexane from vetiver oil extract, in a fumehood.

APPENDIX F

F.1. Carbon dioxide phase diagram

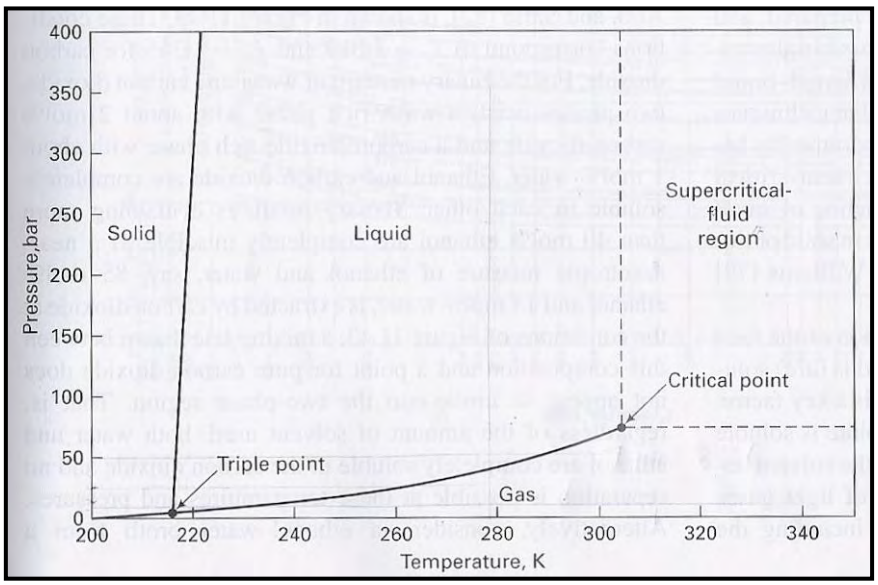


Figure F. 1. Carbon Dioxide Phase Diagram

F.2. Properties of Supercritical Carbon Dioxide

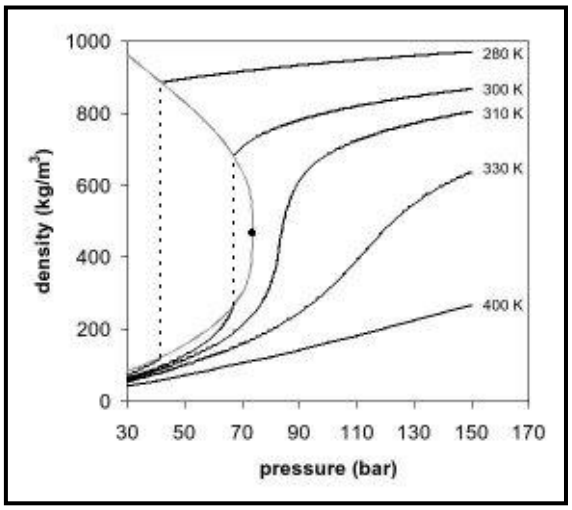


Figure F. 2. Density Behaviour of Carbon Dioxide (Jacobs, 2005)

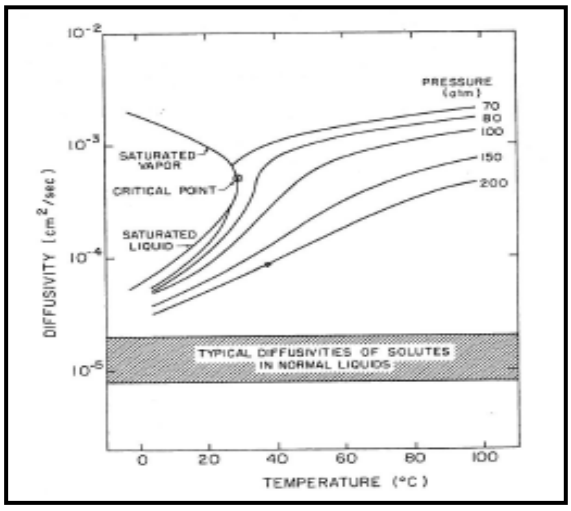


Figure F. 3. Diffusivity Behaviour of Carbon Dioxide (McHugh and Krukonis, 1986)

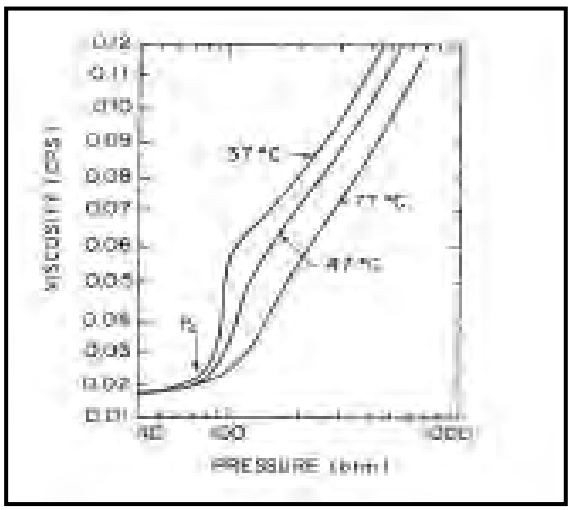


Figure F. 4. Viscosity Behaviour of Carbon Dioxide (McHugh and Krukonis, 1986)

APPENDIX G

G.1. Error analysis for identification of unknowns within the vetiver oil samples

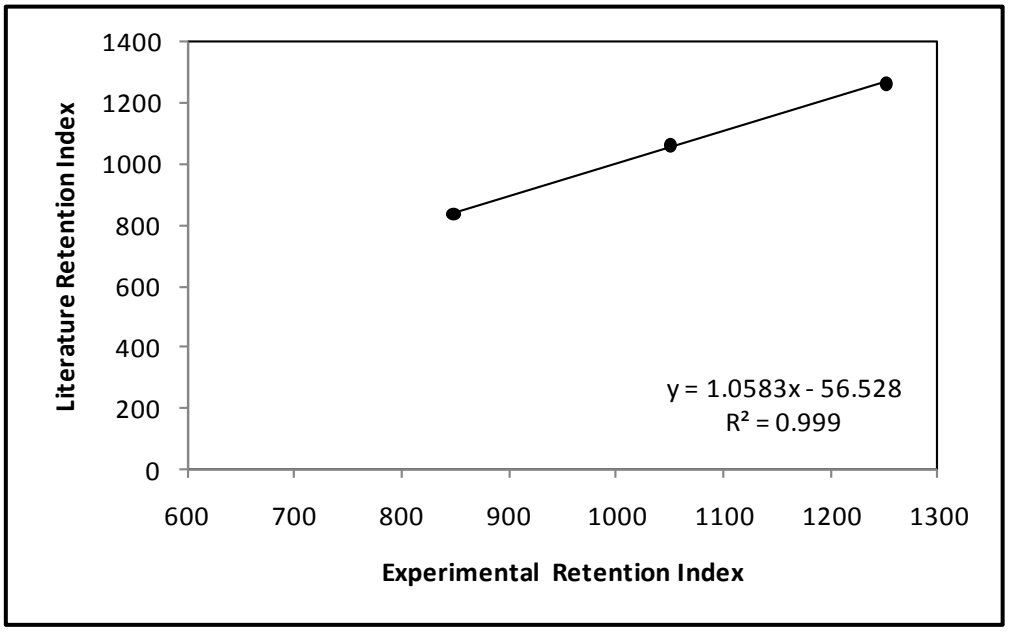


Figure G. 1. Retention index error, literature versus experimentally obtain retention indices.