1 INTRODUCTION

1.1 Background

Humans are and have always been at the quest of conquering nature and its harvest since the beginning of time. Starting from searching and fighting for the basic means of survival they have managed in manipulating and shaping the forces and bounties of nature to their ways and desires. With the advent of ancient civilisation, merchants and explorers have helped in transferring cultural, religious and other practices between distant civilisations. Trade between ancient civilisations flourished and surplus goods were exchanged. Among the goods traded were fragrant plants (*e.g.* Frankincense and myrrh) and spices that could be considered as old as the practice itself [1].

Herbal plants and their essential oils have been used for various purposes since antiquity. The ancient civilisations of Egypt, Rome, Greece, China, and others were known for their use of essential oils as herbal medicines, perfumes, for massaging, as additions to bathing water, and essences were used as offerings to gods and goddesses (*e.g.* burning of Frankincense in temples) [2].

In modern times, essential oils have gained numerous commercial and industrial applications. They are extensively used as ingredients in the perfumery, pharmaceutical and flavour industries. The world trade and consumption in essential oils involves huge amounts of essential oils and money. For example, in 1994 alone, US$ 9 billion worth of flavour and fragrance materials of natural origin were consumed worldwide [3]. Their pleasant fragrance and therapeutic nature have also gained essential oils a wide application in the field of aromatherapy, such as their use as massage oils to relieve different ailments and anxieties or merely as beauty accessories (skin toners). Consequently, many essential oil studies are undertaken which include plant cultivation to increase oil yield, improving oil extraction methods, and the analyses of essential oils for component identification, pattern recognition and quality control purposes.

Essential oils are obtained from different parts of plants, most commonly from leaves, stems and flowers. The oils are very expensive to produce, some more so than others, due to the high labour and capital requirements during cultivation and extraction of the oils and due to the large amount of plant material required to produce sufficient amounts of oil. For example, about 8 million Jasmine flowers are required to produce only 1 kg of Jasmine essential oil [4].

Essential oils consist of a wide variety of compounds including terpene hydrocarbons, alcohols, esters, ketones, aldehydes and acids. The analysis of such complex mixtures calls for the use of various advanced analytical techniques. As early as 1956, gas chromatography has been used for the separation of essential oil components. This technique is able to provide information describing the qualitative and quantitative composition of the volatile and semi-volatile organic components of essential oils. Liberti and Conti were the first to present their work on the separation of monoterpenes in lemon and bergamot oils by using this technique. Later on, from about 1963, gas chromatography-mass spectrometry (GC-MS) came to be widely used as a new separation and identification technique in essential oil analysis. Formacek and Kubeczka also used ^{13}C-NMR in addition to capillary gas chromatography (CGC) to characterise different essential oils of commercial interest [5].

With the development of more analytical techniques, the techniques used to analyse essential oils have diversified over the years. From the coupled chromatographic techniques, in addition to the more dominant gas chromatography-mass spectrometry (GC-MS), supercritical fluid chromatography coupled to capillary gas chromatography has been used for the analysis of essential oils [6]. More recently, comprehensive two-dimensional gas chromatography (GC×GC) has been utilised as an effective tool for the analysis of essential oils [7, 8, 9, 10].

1.2 Approach

This work focuses on the analysis of four South African essential oils by comprehensive two-dimensional gas chromatography. The essential oils analysed are lemongrass, *Artemisia afra*, *Tagetes minuta*, and Bourbon Geranium. The essential oil samples were obtained from different South African commercial farmers through the Department of Agriculture, Conservation and Environment, Lowveld College of Agriculture, Nelspruit. The main aim of this project was to evaluate comprehensive two-dimensional gas chromatography (GC×GC) as a technique for the analysis of essential oils, with special emphasis on the quality control of some locally produced oils. Cost, reliability and ease of data interpretation were among the evaluation criteria.

1.3 Arrangement and Presentation

This dissertation is divided into six chapters. Chapter 1 gives a general introduction. In Chapter 2 a short overview of the origin, definition, use and methods of preparation of essential oils is given. The selected four plants and their essential oils are also separately described in this chapter. Chapter 3 discusses the principles and advantages of multidimensional chromatography and the analysis of essential oils using coupled gas chromatographic techniques. Instrumentation in comprehensive two-dimensional gas chromatography and the optimisation of experimental parameters is dealt with in Chapter 4. Chapter 5 gives the details of the experimental results and the discussions. Concluding remarks are given in the last chapter, Chapter 6. More complete sets of data and GC×GC chromatograms are presented in the appendix.

References

1. http://www.metmuseum.org/toah/hd/ince/hd_ince.htm/02/07/2003.

2. http://www.aromaweb.com/articles/wharoma.asp/25/01/2003.

3. E.A. Weiss, *Essential Oil Crops*, CAB International, 1997, Chapter 1.

4. http://www.aworldofaromatherapy.com/aromatherapy-extraction.htm/03/02/2003.

5. R. Tabacchi and J. Garnero, *Capillary Gas Chromatography in Essential Oil Analysis*, Alfred Huehig Verlag, Heidelberg, 1987, Chapter1.

6. T. Yarita, A. Nomura and Y. Horimoto, Analy. Scie. Vol. 10 (1994) 25 - 29.

7. Jean-Marie D. Dimandja, S.B. Stanfill, J.Grainger, D.G. Patterson, Jr., J. High Resol. Chromatogr. Vol. 23, Issue 3 (2000) 208 - 214.

8. R. Shellie, P. Marriott and C. Cornwell, J. High Resol. Chromatogr. Vol. 23, Issue 9 (2000) 554 - 560.

9. R. Shellie, P. Marriott and P. Morrison, Analytical Chem. Vol. 73, No. 6 (2001) 1336 - 1344.

10. R. Shellie, L. Mondello, P. Marriott and G. Dugo, J. Chromatogr. A Vol. 970 Issue 1 - 2 (2002) 225 - 234.

2 THE ORIGIN, DESCRIPTION, USE, AND PREPARATION OF ESSENTIAL OILS

2.1 What are Essential Oils?

Essential oils are highly concentrated volatile substances extracted from various segments (flowers, roots, leaves, barks, stems, etc) of herbal plants, trees and grasses. The essential oils are accumulated in the oil glands of specialised plant tissues. It is believed that plants utilise essential oils to attract pollinating insects, to repel predators and to protect themselves from diseases. Although almost all plants have odour, not all plants produce commercially viable volatile oils [1]. The quality and quantity of essential oil a plant produces is affected by many factors including the climate, altitude, the fertility, the type of soil where the plants are cultivated and the maturity of the plants. Moreover, the time of the day and season of the year the plants are harvested affects the quality and abundance of the essential oil, which moves around the plant according to both a seasonal and a daily cycle. Individual plant species have their own characteristic rhythm. The quality and availability of an essential oil may also vary from year to year. The other factors that may affect essential oil chemistry are the way the oils are extracted from the plant materials [1, 2, 3, 4].

Though referred to as oils, essential oils are different from cooking or vegetable oils mainly due to their fragrance and volatile nature. Their colour differs from source to source. Their physical state ranges from light liquid having the consistency of water or alcohol (lemongrass, lavender, peppermint, rosemary) to viscous, thick and sticky (myrrh, vetiver) to semi-solid (rose otto) at room temperature. They have a complex chemistry consisting of terpene hydrocarbons, alcohols, aldehydes, acids and esters as major or minor constituents. Due to this complexity, it is impossible to exactly replicate an essential oil in the laboratory [1].

The use of essential oils ranges from the perfume to the pharmaceutical industries. In the field of aromatheraphy[1], they are used as antiseptic (limonene), antiviral (pinene), anti-inflammatory and antibacterial (chamazulene and farnesol from chamomile essence) and anti-rheumatic agents. Esters found in essential oils (linalyl acetate, geranyl acetate) are known for their fungicidal and sedative properties. Although it is true that some ketones like thujone and pulegone are known for their toxicity, there are non-toxic ketones obtained from essential oils with medicinal value. Ketones like jasmone (from jasmine) and fenchone (from sweet fennel) ease congestion and aid the flow of mucus. Alcohols like linalool and geraniol have good antiseptic and antiviral properties [1].

2.2 History of Essential Oils and Aromatherapy

Aromatherapy (aroma = smell, fragrance; therapy = treatment) is an old 'science' dating back as far as the ancient Egyptian civilisation where evidence of the use of herbal medicine to cure illnesses was found in medical papyri (from ~1555 B.C.). Burning of Frankincense was probably one of the earliest ways of using fragrant plants, which was used as an offering to ancient gods and goddesses. The Egyptians also used aromatic herbs for embalming to help preserve the flesh [5].

The Greeks and the Romans also used aromatic herbs for medicinal and cosmetic purposes. The herbal book of the Greek physician, Pedacius Dioacorides, had been used as the Western world's standard medical reference for a very long time. Many of the herbs mentioned in his book are still being used today in aromatherapy [5].

It is believed that the knowledge of fragrant oils and perfumes spread to the Far East and Arabia during the crusades. An Arabian physician called Avicenna or Ibn Sina (980 to 1037 A.D.) is believed to be the first to have used distillation to distil essence of rose. Around this time, the Arabs as well discovered how to distil alcohol, making it possible to produce perfumes without a heavy oily base [5].

[1] **Aromatherapy** is the use of volatile oils, including essential oils, for psychological and physical well being by massage or inhalation [1].

On the other hand, the ancient Chinese might have been practicing their own aroma treatment as early as the Egyptians. Shen Nung's herbal book, the oldest surviving medical book in China, dating back to about 2700 B.C., contains information on over 300 herbal plants [5].

The Indians have also their own traditional medical practice called *ayurveda*, which has been practiced for over 3000 years. In South America the *Aztecs*[2] were known for their plant remedies and the great wealth of medicinal plants at *Montezuma*'s[3] botanical gardens had impressed the Spanish invaders. In the North the American Indians also have their own herbal practice [5].

There are quite a few references to essential oils 'perfume' in the Bible too: "*Take the finest spices 6 kg myrrh, 3 kg sweet-smelling cinnamon, 3 kg sweet-smelling cane, 6 kg of cassia. Add 4 l of olive oil, and make the Sacred anointing oil. (Exodus 30:23-25).*" "*You provided no olive oil for my head, but she has covered my feet with perfume (Luke 7:46)*" etc.

European scientists began researching the effects of essential oils on bacteria and human health late in the 19th century. A French chemist, Rene Maurice Gettefosse, began his research in the medical use of essential oils after he accidentally found the healing powers of lavender oil. In 1937 he published a book about the anti-microbial effects of essential oils and coined the word '*Aromatherapy*'. Around the same time another Frenchman, Albert Couvreur, published a book on the medicinal uses of essential oils [5].

The French medical doctor, Jean Valnet, continued Gattefosse's research on essential oils. The method of applying essential oils to the skin with massage was developed by the French biochemist Margaret Maury. Michine Arcier, a former student and co-worker of both Maury and Valnet, combined all their techniques to create a form of aromatherapy presently used in the world [5].

[2] **Aztecs**: Natives of North America (Mexico).
[3] **Montezuma**: 1466-1520, Aztec emperor of Mexico killed by the Spanish conquistador Cortes.

2.2.1 Aromatherapy and Essential Oil Safety

Used properly, the physical application of essential oils has great benefits in matters of health (massage oils), beauty (in body lotions, ointments, facial toners, perfumes, etc) and hygiene (in soaps, shower gels, shampoos, etc.). Some essential oils can be steam inhaled (*e.g.* eucalyptus oil) to help relieve colds and influenza or for relaxing overworked muscles. Essential oils are applied to the skin after diluting them in carrier oils, which are pure vegetable oils like sweet almond oil, apricot kernel oil, sesame, avocado, grape-seed oil, etc., to help their absorption by the body. Additionally, essential oils are widely used in room refreshing (rose, lemon) and insect repellent (citronella, lavender, peppermint) sprays [5].

To enjoy all the benefits of essential oils and to avoid the risks involved in their use, the following is generally recommended [5]:

- Essential oils should never be used undiluted. Some oil constituents may have a negative effect on health.
- Due to possible allergic reactions, essential oils have to be used with caution. The use of some essential oils in aromatherapy may have dire consequences. Examples include onion, wormwood, pennyroyal, camphor, horseradish, wintergreen, rue and bitter almond essential oils.
- Some essential oils should be avoided during pregnancy (*e.g.* lemongrass) or by persons with asthma or epilepsy. Essential oils should never be used on open wounds or taken orally in such cases.
- Essential oils are flammable and constitute a fire hazard.

2.3 General Description and Use of the Four Essential Oils Analysed in this Project

2.3.1 Lemongrass (*Cymbopogon citratus & C. flexuosus*)

Lemongrass, from the Gramineae (aromatic grasses) family, is native to India [6]. It is a vigorous grass, which may be harvested within six months after planting and may be cut three to four times a year. Lemongrass essential oil, obtained mostly by distillation [7], has a lemony sweet smell, dark to amber-reddish colour and watery viscosity [1].

The essential oil is widely used industrially and it is found in cosmetics such as hair conditioners, facial water, lotions, deodorants and soaps [8]. Added to water or vinegar and sprayed in the air, along walls and floors, or on pets, it acts as an insect repellent and attacks fungi by discouraging mould growth. An antiseptic wash helps to improve skin infections and sores. The numbing of nerve endings, which dulls the intensity of pain that reaches the brain, caused by lemongrass essential oil makes it useful to relieve headaches, indigestion, pain, rheumatism, and nervousness. The scent reduces irritability and drowsiness [7]. Blended with other essential oils, such as lavender, basil, and jasmine essential oils, it is used in massage or diluted in bath to help cellulite, digestive problems, over exerted ligaments and as a general tonic [1]. But the oil should only be used under the guidance of a professional aromatherapist to avoid any side effects that might occur [6].

Traditionally, lemongrass is used widely for the treatment of different ailments. In Tanzania and Kenya an infusion of lemongrass is taken as tea for fevers and jaundice, to treat acne, skin infection, and ringworms. It is also used as a body wash and to perfume the body. The Zulus used the plant juice for ritual cleansing washes. The Zulus also used the sap from the cooked roots to settle the stomach [9].

Fresh lemongrass leaves have been traditionally used in Thai, Vietnamese and Caribbean cooking for many years. The herb is frequently used in curries as well as in seafood soups. Its light lemon flavour blends well with garlic, chillies and ciliantro. But due to its strong odour it is normally used in small amounts [8].

2.3.2 Bourbon Geranium (*Pelargonium capitatum x P. radens*)

Geranium, from the Geraniaceae family, constitutes a large number of flowering plants (~700), of which only about 10 varieties and some hybrids, like the one analysed in this work[4], produce viable quantities of essential oils. It is a hairy perennial shrub; stands up to about one meter high, with pointed leaves serrated at the edges. Originally, the plant was native to South Africa, Reunion, Madagascar, Egypt and Morocco before its introduction to other countries worldwide [10].

The essential oil is extracted from the leaves and stalks mostly by steam distillation. The essential oil analysed here has a slightly light green colour, watery viscosity and lemony fragrance. Geranium oil has quite a number of therapeutic properties and can be used to help treat: acne, bruises, burns, cuts, dermatitis, ulcers, diabetes, colds, flu [5], etc. It is used as massage oil and diluted in bathing water. The oil blends well with other essential oils like cedar wood, bergamot, basil, jasmine, lavender, orange and rosemary. It has also insect repellent properties. Due to its hormone balancing behaviour, the essential oil of pelargonium is not recommended for pregnant women [1].

2.3.3 *Tagetes minuta* (Kakiebos)

Tagetes minuta, from the Compositae (daisy family) [6], is an herbaceous plant/weed about 1 m tall with small yellow flowers and a very strong smell. Other names of *Tagetes minuta* include Mexican Marigold, tall Khaki weed (English), 'Kakiebos', and 'Lang-kakiebos' (Afrikaans). *Tagetes minuta*, believed to be native to Africa [5], is grown in East and South Africa, South America, and Australia. Grown in the wild it is a problematic weed of pastures and numerous crops. *Tagetes minuta* seeds have an unpleasant odour and can reduce the value of grain harvest when it is a contaminant [11].

Commercially, it is grown for its oil, which has a heavy, pungent, sweet smell and gold-yellow to reddish-amber colour. It is medium in viscosity that turns thick and even gel-like if exposed to the air for a long time. *Tagetes minuta* oil is extracted (distilled) from leaves,

[4] A cross breed between *Pelargonium capitatum* and *Pelargonium radens*.

stalks and flowers picked when the seeds are just starting to form. The oil is used in French perfumes and for flavouring numerous food products. As a food flavourant it is used in cola and alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatines, puddings, condiments, and relishes [12].

Tagetes minuta is rich in many secondary compounds, including acyclic, monocyclic, and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes, and aromatic compounds. There is evidence that the secondary compounds in *Tagetes minuta* are the effective deterrents of numerous organisms, including fungi, bacteria, round worms in general, trematodes, nematodes and numerous insect pests through several mechanisms [12].

Traditionally, *Tagetes minuta* is best known for its skin care remedies. Ailments such as calluses, bunions, and fungal infections are treated with an infusion from the leaves and flowers made into a wash or cream applied to the area [8]. Crushed mature *Tagetes minuta* leaves and flowers can be used as general insect repellent and fungicidal agents. Ants, aphids, blowflies, caterpillars, flies, fleas, maggots, mosquitoes and termites are repelled if soil or surfaces are sprayed with a *Tagetes minuta* juice or if a freshly crushed plant part is dug into the soil[5].

Keeping in mind all its uses, *Tagetes minuta* is a very powerful oil and should be used sparingly. It should be avoided during pregnancy. It should not be used on sensitive skin as it may cause photosensitivity and some form of dermatitis [1]. *Tagetes minuta* oil should never be taken orally [5].

2.3.4 African Wormwood (*Artemisia afra*)

Artemisia afra, a well known medicinal plant in Eastern and Southern Africa, is also known by other common names like African Wormwood (English), 'Wilde Als' (Afrikaans), 'Lanyana' (Sotho, Tswana) and 'Umhlonyane' (Xhosa, Zulu). African Wormwood, which got its name from its ability to get rid of worms, is a perennial drought resistant, feathery,

[5] Mexican Marigold (*Tagetes minuta*)- author anonymous.

grey-green shrub. It is about 1 – 2 m tall with small yellow flowers and can grow almost in any soil [13]. The essential oil has a light yellow colour, watery viscosity and strong odour.

Traditionally, the infusion of *Artemisia afra* was used as a lotion to bathe hemorrhoids, and with a hot bath to bring out measles. In Tanzania a weak infusion of the leaf was used to treat colic. Warmed leaves were used as a poultice for pimples, boils, mumps, and sprains [8]. In South Africa, *Artemisia afra* is traditionally used as a remedy for chest problems, coughs, colds, influenza, loss of appetite, malaria and other ailments. Its volatile oil shows antibacterial, narcotic, analgesic and antihistamine activities [14].

2.4 Essential Oil Preparation Methods

Essential oils are very expensive to produce, some more so than others, due to the huge labour, expertise and capital investment requirements during cultivation and extraction of the oils. As already indicated, essential oils are obtained from different herbal plants and plant parts. A plant may give oil from one or more parts, but the amount of oil that might be produced may not be equally valuable both in quality and quantity. The method of extracting the essential oils from a specified plant material may also have an effect on its final composition [15].

Commercially, essential oils are obtained using a variety of methods. Distillation, Solvent Extraction, and Supercritical Fluid Extraction (CO_2 extraction) are the three main essential oil isolation methods employed in industry and will be dealt with in the sections to follow.

2.4.1 Distillation

Distillation is the oldest and most widely used extraction technique in the commercial production of essential oils due to its cost effectiveness. It is done in a variety of forms *viz.*: water distillation, steam distillation, hydro diffusion, water-steam distillation, cohobation, and rectification. The extraction of essential oils using distillation requires great care as prolonged heating may induce chemical changes that can damage the 'true' essence and nature of the essential oils [16].

(a) Extraction of Essential Oils by Water Distillation

In this process the plant material is completely immersed in water and the mixture is boiled. After some time the condensed mixture is allowed to cool and settle down, whereby the water-oil liquid mixture is separated from the solid plant residue. The essential oil floats on top of the hydrosol[6] and the two are separated for their destined use [16].

Essential oil sources rich in esters should not be extracted by this method as extended exposure to hot water will start to break down the esters to their respective alcohols and carboxylic acids, mostly acetic acid. To limit damage to heat sensitive essential oils, distillation can be performed under reduced pressure. This method is more effective for oils extracted from tough materials like roots, wood, or nuts [16].

(b) Extraction of Essential Oils Using Steam Distillation

In steam distillation, pressurised steam is forced through the plant material to help release the fragrant molecules from the pockets in which the oils are present. The molecules of these volatile oils then evaporate and escape from the plant material into the steam. The pressurised steam is kept hot enough (~ 100°C) to release the oil from the oil glands, yet not too hot to damage the plant material or the essential oil. The steam containing the oil is then passed through a cooling system, condensed, and decanted to give the oil and hydrosol [16, 17].

Some of the drawbacks of this method are [18]:

- The steam released from the bottom of the still[7] does not disperse very well, making the process less efficient.
- A long time is required to completely distil the oil, which increases the time the plant material is exposed to heat.
- It is costly as it takes lots of energy to produce the steam and for cooling the products.

---

[6] A **Hydrosol** or **floral water** is the water that remains after extracting an essential oil *via* steam or water distillation, which is then used in skin care and bathing products or as light colognes or body sprays.

[7] **Still**: An apparatus for distilling liquids, such as alcohols, consisting of a vessel in which the substance is vaporised by heat and a cooling device in which the vapour is condensed.

(c) Extraction of Essential Oils Using Hydro-Diffusion

Hydro-diffusion differs from steam distillation in that the steam is introduced into the plant material from the top down instead of from the bottom up as in steam distillation. The oil containing steam mixture condenses below the area in which the botanical material is held in place by a grill, a grating of metal or wood used as a barrier. Less steam requirement, shorter processing time and higher oil yields are some of the advantages of hydro diffusion [16].

(d) Extraction of Essential Oils By Water and Steam Distillation

This technique is a combination of both water and steam distillations. The plant material is immersed in a still with a heat source, and additional steam is fed into the mixture [16]. This method is best suitable for leafy material, but has little use for tough materials such as woods, roots and seeds [19].

(e) Extraction of Essential Oils by Cohobation

Cohobation is a method employed to distil and re-introduce water-soluble oil components into the essential oil after the oil has been extracted by water distillation. For example, when Rose oil is extracted using water distillation, one of its components, ethyl phenyl alcohol, dissolves into the water and needs to be redistilled from the water and re-introduced to the essential oil to make the oil 'complete' [16].

(f) Extraction of Essential Oils Using Rectification

Rectification is a process of re-distillation used to refine and purify extracted essential oils that have water, resinous matter or solvents as impurities. It is done either in steam or under vacuum. For example, Eucalyptus oil is re-distilled to create standard quality oil [16].

2.4.1.1 Problems Associated with Distillation

The length of the distillation period affects the composition of most essential oils. This is especially true for oils that are obtained from seeds, barks, or any other plant parts with a hard resistive barrier between the oil glands and the surface in contact with the boiling water. At the first stage of distillation higher boiling oxygenates are liberated more easily than lower boiling hydrocarbons; in the process of distillation the boiling water breaks into the plant tissue, dissolves the water-soluble oil components and carries them to the outer surface to be vaporised. This process called hydro-diffusion continues until all the essential oil is removed. The hydrocarbons remain associated with the plant material longer than the oxygenated oil constituents because of the latter's lower water solubility. As a consequence of the process of hydro-diffusion, essential oil components are distilled in the order of their water solubility and not in the order of their boiling point [15].

Non-volatile fats that might be present in essential oil materials have the power of retaining essential oil components, preventing complete distillation of the oil. This is more the case with hydrocarbons that show a greater affinity for the fatty component than the oxygenated compounds [15].

The process of distillation also introduces new constituents due to chemical changes that may occur during the extraction procedure, including isomerisation, saponification, polymerisation or degradation. One of the main effects observed during essential oil extraction by distillation is the effect of temperature and pH, which makes some oil constituents prone to undergo rearrangement or hydrolysis reactions. Increased temperature and decreased pH (increased acidity), for example, transform labile components like linalyl and α-terpinyl acetate to their corresponding alcohols: linalool and α-terpinol [15]. Linalool, which is present in almost all essential oils, rearranges to terpineol during steam distillation [20], as shown in figure (2.1). The presence of trace metals in plants has also been observed to cause rearrangement of some compounds to their isomers. For example, *cis*-dihydrocarvone isomerises to its *trans* isomer during distillation [15].

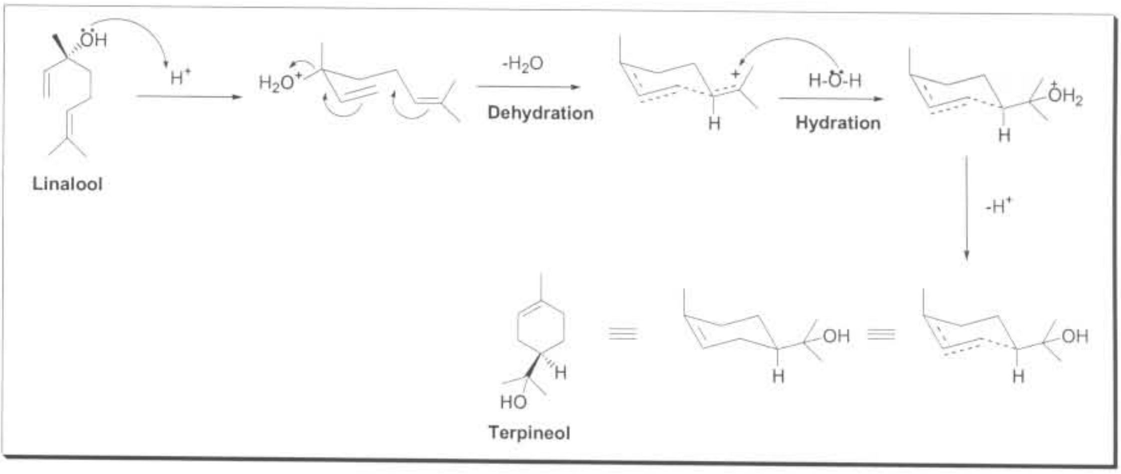

Figure 2.1 The re-arrangement of linalool to terpineol during steam distillation [20].

By contrast, the oil enclosed in glandular hairs is easily liberated because the only barrier, the outer membrane or the cuticle, simply bursts open when the distillation water reaches boiling point. Therefore, the above-mentioned problems of distillation are theoretically least expected to act in cases where the oil is extracted from leaves, flowers or any other soft plant material [15].

2.4.2 Solvent Extraction of Essential Oils

Essential oils can also be extracted by solvent extraction. Solvent extraction, which is most suitable for delicate and heat sensitive fragrant oils [16], is done by either a Soxhlet extraction or by mixing the plant material and solvent in an Erlenmeyer flask [15]. Different solvents including hexane, acetone, petroleum ether, ethanol, and methanol [16] are used for this extraction method. During solvent extraction the solvent dissolves a range of substances from the plant including non-aromatic waxes, pigments, and highly volatile aromatic molecules. The solution containing both solvent and soluble plant material is filtered and the filtrate is subjected to low-pressure distillation to remove and recover the solvent for further use. The remaining waxy mass called the *concrete* contains the volatile essential oils [19].

To remove the waxy materials, the concrete is warmed and stirred with alcohol (usually ethanol). The heating and stirring process breaks the concrete into minute globules. The alcohol soluble oil components separate out efficiently with a certain amount of alcohol-soluble waxes. Agitating and cooling the solution at low temperatures helps to precipitate out the waxes. Finally, the solution is cold filtered to get the pure essential oil called the *absolute* [19].

The use of low boiling solvents for the extraction of oils has an advantage over distillation, as the temperature remains relatively low during most of the process. Solvent extracted essential oils have more of a natural appearance, free of distillation-induced artefacts. However, besides the need for further purification to remove undesired waxes and pigments, solvent extraction may also introduce artefacts. Artefacts produced from solvent (acetone, petroleum ether) and essential oil component (non-terpenoids) interactions may be observed in the volatile extracts. Alcohol extraction might also give rise to the formation of artefacts by esterification, etherification, and acetal formation [15].

2.4.3 Supercritical Fluid Extraction (SFE) of Essential Oils

Supercritical fluid extraction employs the unique properties of some substances (water, CO_2, butane, etc.), which give a supercritical fluid with gaseous and liquid properties at certain temperatures and pressures called the critical points. Supercritical fluids have good solvent properties similar to those of liquids as well as the transporting properties of gases. Carbon dioxide, due to its low cost, low critical pressure and temperature and low toxicity is the most widely used supercritical fluid. It forms a supercritical fluid at about a pressure of 73.8 bars and 31.1°C. In CO_2 extraction, the plant is placed in a stainless steel tank and CO_2 is injected into the tank. Under the appropriate pressure and temperature the CO_2 is liquefied and acts as a solvent to extract the essential oils from the plant materials [16]. The extraction capability and capacity (dissolving power) of the inert CO_2 fluid can be manipulated by regulating the system's temperature and pressure (fluid density) [21].

The advantage of CO_2 extraction over solvent extraction is that it doesn't chemically interact with the essential oils [18] and no solvent residue remains. At normal pressure and temperature the CO_2 reverts to the gas phase and evaporates leaving the pure essential oil behind. Compared to distillation, it is gentler and thermally labile compounds can be extracted easily [21] as high temperatures are not employed. Supercritical CO_2 extraction helps not only to extract oils which do not usually yield to other methods of extraction (*e.g.* Rose Hip Seed, Calendula), but also provides more intensely scented essential oils since more of the fragrant chemicals are released through this process [19].

As already stated, the process of supercritical extraction requires heavy-duty stainless steel equipment and hence high capital investment is needed in using this extraction method [16]. The other aspect that needs consideration in this extraction process is the relative acidity of CO_2 (~ 4.5), which might change the composition of some oils. Therefore, oils with a high content of acid sensitive compounds, such as terpenes, are not suitable for supercritical CO_2 extraction [22].

References

1. http://www.essential-oil.org/shop/essential.htm/25/01/2003.
2. A. Gil, C.M. Ghersa, and S. Leicach, Biochem. Systematics and Ecology 28 (2000) 261 - 274.
3. E.H. Graven, L. Webber, G. Benians, M. Venter, and J.B. Gardner, J. Ess. Oil Res. 3 (1991) 303 - 307.
4. P. Boruah, B.P. Isra, M.G. Pathak and A.C. Ghosh, J. Ess. Oil Res. 7 (1995) 337 - 338.
5. http://www.aromaweb.com/articles/wharoma.asp/25/01/2003.
6. Lisa Chidell, *Aromatherapy: A Definitive Guide to Essential Oils*, Hodder and Stroughton, Headway, 1991.
7. http://www.antiagingchoices.com/Aromatherapy/lemongrass_oil.htm/27/01/2003.
8. http://www.naturedirect2u.com/Essential%20oils/lemongrass.htm/27/01/2003.
9. http://www.africasgarden.com/Library.htm/27/01/2003.
10. http://purelinatural.com/GeraniumOil.html/05/02/2003.
11. http://pi.cdfa.ca.gov/weedinfo/TAGETES2.html/27/01/2003.
12. http://www.hort.purdue.edu/newcrop/proceedings1993/v2-649.html/27/01/2003.
13. http://www.gardening.worldonline.co.za/0565.html/05/02/2003.
14. http://www.spirit-web.com/Artemisia%20Afra.asp/05/02/2003.
15. Arthur Koedam, *Capillary Gas Chromatography in Essential Oil Analysis*, Alfred Huehig Verlag, Heidelberg, 1987, Chapter 2.
16. http://www.essentialoils.co.za/distilation.html/03/02/2003.

17. http://www.oneplanetnatural.com/distillation.html/03/02/2003.

18. http://www.aromawerks.com/Distillation.html/03/02/2003.

19. http://www.naturesgift.com/extraction.html/03/02/2003.

20. P. Teisseire, *Capillary Gas Chromatography in Essential Oil Analysis*, Alfred Huehig Verlag, Heidelberg, 1987, Chapter 7.

21. http://www.sunny.vemt.bme.hu/sfe/anglo/supercritical.html/05/02/2003.

22. http://www.carnagepro.com/pup/Mic/tane/other_methods.html/05/02/2003