ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Extraction and Characterization of Essential Oil from Rosemary Leaves.

A Thesis Submitted to the School of Chemical and Bio-Engineering Presented in Partial Fulfillment of the Requirements of the Degree of Masters of Science in Chemical Engineering (Process Engineering Stream)

By
Solomon Hailemariam

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List of abbreviations

AAIT	Addis Ababa Institute of Technology
AV	Acid Value
A.O.A.C.	Association of Analytical Chemistry
ANOVA	Analysis of variance
ASTM	American Society of Testing and Material
EO	Essential Oils
EEG	Electroencephalogram
EDA	Electro – Dermal Activity
FID	Flame Ionization Detector
GC	Gas chromatography
GC-MS	Gas chromatography Mass spectroscopy
HHV	High Heating Value
ISO	International Standards Organization
IV	Iodine Value
NIST	National Institute of Standard and Technology
MSD	Mass Selective Detector
SSE	Sum Square Errors
SFE	Supercritical Fluid Extraction

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Abstract

Rosemary (*Rosmarinus officinalis* L.) extracts enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups and they also possess innumerable applications starting from markers for plant identifications to bases for semi-synthesis of highly complex molecules. Therefore, the aim of this research is extraction and characterization of rosemary essential oil (*Rosmarinus officinalis*) using steam distillation method.

In this research, rosemary leaves were collected from Sebeta region (town and separate wereda in central Ethiopia). An integrated procedure for steam distillation extraction followed by volatiles sampling and characterization from the leaves of the rosemary was implored. The effect of different process parameters, such as pre-distillation drying conditions and extraction time on the extraction yield was investigated. The parameters taken up for the study like the pre-distillation drying conditions and extraction time were found to significantly affect the extraction yield and quality of the essential oil. Increase in yield was observed when increase the extraction time and pre-distillation drying conditions. The essential oil yield was found to vary from 0.378 to 1.137% under different method of drying and extraction time. Maximum yield was observed from sun dried rosemary leaves at 120 min of extraction time. The volatiles rosemary essential oil in vapour form released from the leaves were condensed and characterized their physical and chemical properties and found within the recommended range. The components of rosemary essential oil was determined using Gas Chromatography-Mass Spectroscopy, and eight major components were identified. Monoterpene hydrocarbons were found to be predominant. The major components were α-pinene (12.08 %), camphor (9.14 %), camphene (2.14 %), bornyl acetate (6.79 %), borneol (5.07%), and α-terpineol (3.08%). Therefore, pre-distillation drying and extraction time have greater effect on the quality and quantity of rosemary essential oils.

Key words: *Rosmarinus officinalis L*; rosemary; essential oil composition; extraction; steam distillation.

1. Introduction

1.1. Back ground

Rosemary (*Rosmarinus officinalis* L.) is an aromatic medicinal and condiment herb or plant that belongs to the Family Labiate. It can be cultivated in arid and rocky areas and can grow in calcareous rich soil. Rosemary is a wild shrub having one to two meters high and with small and persistent green leaves. It is relatively rich in essential oil, which can be extracted from the flower itself or from the leaves, where most of oil glands are located. However, the highest quality of essential oil is obtained from the leaves. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent. Major constituents described for the oil are α-pinene, 1, 8-cineole and camphor (Petra et al., 2009).

Rosemary essential oils are recovered by accepted procedures, such that the nature and composition of the product is as nearly as practicable, unchanged by such procedures. It specifies clearly that the nature and composition of the oil must be unchanged by the process of extraction, which is one reason why steam is an appropriate method of extraction. Furthermore, because steam distillation has been the extraction method for most essential oils, the market accepts steam distilled oil as normal oil. Rosemary essential oils are used widely by the pharmaceutical and cosmetic/ perfumery industries as well as in aromatherapy and alternative medicines.

Essential oils have some distinctive characteristics, which make them a very valuable commodity with many industrial uses and applications. Their aromatic value enables them to be used as flavoring in both the food and beverage industries. These oils are also widely used in both the cosmetic and pharmaceutical industries. With such applications, there is a huge demand for rosemary essential oils worldwide and hence they have been traded internationally for several centuries. There is hence a need to improve the quality and quantity of the essential oils produced as they have a very competitive and profitable market worldwide. The chemical composition of the essential oils is important in determining their quality and consequently price in the market. It is therefore important to note and understand some of the parameters such as temperature, pressure, drying conditions, particle size and time of extraction may affect the quality and yield of essential oil.

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method of extraction is steam distillation, but as technological advances are made more efficient and economical methods are being developed. These include methods such as solvent extraction, supercritical fluid extraction, cold pressing and microwave extraction. The suitability of extraction method varies from plant to plant and there are significant differences in the capital and operational costs associated.

The post-harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold. Drying has been one of the most important processes in pre-processing of agricultural products. Aromatic plants are often dried before extraction to reduce moisture content. The aim of drying is to reduce the moisture content of the product from actively growing in the field to a level that prevents deterioration of the product and allows storage in a stable condition. Proper drying of medicinal plants is fundamental to the achievement of a high quality product. A literature search was undertaken on effects of different methods of drying on essential oil content and chemical composition of the essential oil plants. The results showed that drying method had a significant effect on oil content and composition of aromatic plants. Also duration of essential oil extraction affected on the quantity and quality of essential oil. (Jamshidi et al, 2004) reported that essential oil percentage and essential oil component of fennel were affected by duration of essential oil extraction.

1.2. Statement of the problem

The increasing importance of essential oils as pharmaceutical and aromatherapy aid besides their traditional role in cosmetics not only as potent ingredient but also as a fragrance donor has opened up wide opportunities for global marketing. The global market for essential oils size has been estimated at USD 6.0 billion in 2015, with an annual growth rate of 8.26 percent. It is projected that the value of the global market for essential oil products would reach USD 13.94 billion by the year 2024. (Grand Viewer, 2016).

Ethiopia is endowed with a diversity of flora. Most of which has remained unexploited. Its application in antioxidant, aromatherapy, perfumery, soap and other related industries are limited due to lack of adequate research on the chemical and biological potential of its raw materials. Among these plants, which are not well known about their valuable uses, rosemary is one of the most useful plants and so far, its many uses are not discovered.

Currently Ethiopia is importing phenolic compounds from abroad for chemical and pharmaceutical uses although the main constitute of rosemary oil was used as a raw material in different chemical industries. Rosemary (*rosmarinus officinalis* L.) is considered one of the most important sources for the extraction of phenolic compounds with strong antioxidant activity. Rosemary extracts, enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups but they also possess plenty of other beneficial effects like flavor in cosmetics, antimicrobial, antiviral, anti-inflammatory, anti-carcinogenic activities and is also known to be an effective chemo-preventive agent. They are also used for aromatherapy, a natural remedy to cure psychological stress and poor physical conditions, and are also used as food flavorings to be added to beverages, confectionery and other processed foods, and as cosmetic fragrances for perfume products or toiletries.

The exchange obtained from exporting or stopping importing this phenolic material plays a great role in economic growth of the country. The chemical and pharmaceutical importance of rosemary is significant and needs to invest more on the mass production of this plant.

1.3. Objectives of the research

1.3.1. General Objective

The general and the main objective of this research is extraction and characterization of essential oil from rosemary (*Rosmarinus officinalis*) leaves.

1.3.2. Specific Objectives

The specific objectives are:

i. Extraction of essential oil from rosemary leaves using steam distillation.

ii. Investigation on the effect of extraction time and drying conditions of rosemary leaves on product yield.

iii. Determination of optimum conditions for the extraction of essential oil

iv. Characterization of extracted rosemary essential oil.

1.4. Significance of the research

Ethiopia currently imports essential oil for the pharmaceutical and cosmetics industries. This project seeks to provide import substitution by enabling the development of small scale process plants to extract essential oil from rosemary using steam extraction methods. The technology will be transferred to interested private sectors or any other agencies. The government's poverty alleviation program will be well supported by proliferation of these small scale process plants through Ethiopia through the technology station program.

2. Literature Review

2.1. General description

Essential oils are volatile oil of plant origin. Essentially the oil is named the plant from which it is derived. The oil can be recovered from plants by various methods, chiefly among them are steam distillation (including water and steam distillation) expression, solvent extraction, effleurage and maceration. These are all physical processes. Essential oils are derived from rosemary, almond (bitter) bay, beryamond, clover eucalyptus, lavender, lemon, orange, peppermint and rove . Physical processes mentioned above isolate most of these. However, some of these oils such as bitter bay and certain rose oils are steam distilled only after some fermentation has occurred.

Essential oils are produce in the protoplasm of the plant cells. They are plant excretion compounds, which do not take place in the metabolism of the plant. They are rich in energy and chemically very active. Therefore, it is quite uncommon to understand why plants expel such an amount of energy, without making use of it. The oils are stored in the form of micro droplets in the gland of the plant. These droplets diffuse slowly through the walls and evaporate, consequently filling the air with the perfume (Industrial Technology Institute (CISIR) & National Science Foundation, 1999). The tropics with greatest solar energy have the most odoriferous plants. Young plants usually produce more oil than old ones. This is due to the continued evaporation of the higher fractions of the oil. Old plants are richer in more resinous and darker oil. In general, essential oils are insoluble in water but soluble in organic solvents, although in some cases (for example, rose water and dragger flower water) enough of the oil may be dissolved in water to give intensive odor to the solution. An estimated 3000 essential oils are known of which approximately 300 are of commercial importance.

2.2. Uniqueness of essential oils

In early work, the term "essential oils" was defined as the volatile oils obtained by the steam distillation of plants. This definition was clearly intended to make a distinction between "fatty oils" and the oils, which are easily volatile. Gradually with the advance of science came improvements in the methods of preparing the oils, and parallel with this development a better knowledge of the constituents of the oils was gained. It was found that the oils contain many classes of organic substances with varying volatility. Although a list of all the known oil components would include a variety of chemically unrelated compounds, it is possible to classify these into four main groups of essential oils (Guenther, 1960):

- ✓ Terpenes, related to isoprene
- ✓ Straight-chain compounds, not containing any side branches
- ✓ Benzene derivatives
- ✓ Miscellaneous

Essential oils are different from other oils by their properties:

A. Volatile

Essential oils are the volatile fragrant components from various indigenous and exotic plants which have been traded internationally for several centuries (Yesenofski, 2005). All true essential oils are secondary metabolites of plant products and in some instances the oil extracted from one part of the plant is different from that extracted from other parts (Learmoth et al., 2002).

B. Aromatic

Essential oils are highly aromatic and therefore, many of the benefits can be obtain by simply inhaling them. This can be done by breathing in the fragrance from the bottle, or they can be diffused into the room.

Essential oils, when diffused, can be the best air filtration system in the world.

They will:

- ➢ Purify the air by removing metallic particles and toxins from the air
- ➢ Increase atmospheric oxygen
- ➢ Increase ozone and negative ions in the house, which inhibits bacterial growth
- ➢ Destroy mold, cigarettes and animal odors
- ➢ Fill the air with a fresh, herbal aromatic scent (Becker, 2005).

C. Penetrating characteristics

The penetrating characteristic of essential oils greatly enhances their ability to be effective. Essential oils will penetrate into the body when applied to the skin. Essential oils rubbed into the feet will be distributed to every cell in the body in minutes. They will even penetrate a finger or toe nail to treat fungal infection underneath. Other vegetable oils do not have this propensity to penetrate (Becker, 2005).

D. High frequency

The effectiveness of essential oils is sometimes also described in terms of frequency. It has been reported that the human body has an electrical frequency and that much about a person's health can be determined by frequency. In 1992, Bruce Tainio of Tanio Technology, an independent division of Eastern State University in Cheny, Washington, built the first frequency monitor in the world. Tainio has determined that the average frequency of the human body during the day time is 62-68 MHz. (a healthy body frequency is 62-72). When the frequency drops, the immune system is compromised. If the frequency drops to 58 MHz, cold and flu symptoms appear, at 55 MHz, diseases like Candida take hold, at 52 MHz, Epstein bar and at 42 MHz, cancer.

According to Dr. Royal R. Rife, every disease has a frequency (Becker, 2005). He found that certain frequencies can prevent the development of disease and that others would destroy disease. Substances with higher frequency will destroy diseases of a lower frequency. The study of frequencies raises important questions, concerning the frequencies of substances we eat breath and absorb. Many pollutants lower healthy frequency. Processed canned food has a frequency of zero. Fresh produce has up to 27 MHz. Essential oil start at 52 MHz and go as high as 320 MHz, which is the frequency of Rose oil. Clinical research shows that essential oils have the highest frequency of any natural substance known to man, creating an environment in which disease, bacteria, virus, fungus, etc., cannot live (Becker, 2005).

2.3. Uses of essential oils

2.3.1. General uses of essential oils

According to ancient Egyptian hieroglyphics and Chinese manuscripts, priests and physicians were using essential oils thousands of years before Christ to heal the sick. These are the oldest form of medicine and cosmetic known to man and were considered more valuable than gold. There are 188 references to oils in the Bible. The wise men brought the Christ child gold, frankincense and myrrh. Current clinical research shows that frankincense oil contains a high concentration of immune stimulating properties. In Exodus, God gives Moses the formula for "holy anointing oil" (Exodus 30:22-25). Some of the precious oils which have been used since antiquity for anointing and healing the sick are frankincense, myrrh, galbanum, hyssop, cassia, cinnamon and spikenard. Science is only now beginning to investigate the incredible healing substances found in essential oils (Becker, 2005).

Essential oils have a wide range of uses:

- As an important sources of natural flavors and are used extensively in the agricultural and food sector.
- As fragrances and are used in perfumed, beauty products, deodorants, soap and detergents.
- As components in pharmaceuticals, antiseptic, and aromatherapy products.

2.3.2. Major uses of essential oils

2.3.2.1. Fragrant uses

Currently fragrant of essential oils are mainly used by the cosmetic industry. Smell has quite an effect on the human body and its nervous responses. The hippocampus, formic, cingulated gyrus, thalamus, mamillary bodies, amygdale and olfactory bulb represent the limbic system in the human body. And although this might sound far too technical to be interesting, it is fascinating to know how the sense of smell actually happens and the effect it has on us all. Before the term "limbic system" was used, the system was referred to as the *rhinencephalon* or "smell brain" and is considered as one of the oldest systems in the human body. When a smell enters the nasal cavity, it meets up with over 50,000,000 receptor neurons which are located in the upper part of the nose and nasal septum, and the receptors are specialized in such a way that certain react to certain smells. These receptors then convert the presence of a smell into a message which is sent to the olfactory bulb (which is seen as part of the forebrain and its main sensory input) where the processing of the smell is started and then passed onto other areas of the brain which control emotions, behavior as well as basic thought processes. Some distant memories, for instance from childhood, can only be recalled by smell, and this fact underscores the importance and significance of smell for our mental life and well-being.

2.3.2.2. Brainwaves

When measuring brainwaves by means of an Electro Encephalo Gram (EEG), it has been noted that was an increase of alpha waves when smelling lavender oil, which is assumed to be a relaxant fragrance. These test results did however vary when test subjects were in different states of arousal or relaxation. When using alerting odours, such as jasmine, an increase in brain activity was noted. On the other hand, certain odours such as nutmeg, mace extract and valerian oil reduced systolic blood pressure and stress. Interestingly an odour need not be very strong to have an effect on the body. With Electro-Dermal Activity (EDA) testing (where the electrical current between two points of the skin is measured) it was also found that slower currents occurred with relaxing odours such as bergamot and lavender were inhaled.

2.3.2.3. Hedonics

It must however be kept in mind that hedonics play a large part in interpreting the results of such tests. Hedonics is the personal degree of pleasantness that a person would place on a specific odour or smell. The limbic system is more than just a part of our smelling mechanism: it is an integral part of man in the wider context. Since it has a direct and indirect influence on so many of our body systems, including the regulation of the endocrine and visceral effect or mechanisms and the resultant patterns of behavior and motivation, it therefore implies that our sense of smell is more than simply a coping mechanism, but fulfils its own regulatory work as well. Although much scientific research has been done on how odors, and in particular the fragrances of essential oils, influence the physiology of the body, much is still unexplained and calls for further investigation.

2.3.2.4. Medicinal uses

Rosemary oil is often used for indigestion, relieving flatulence, stomach cramps, constipation, and bloating. Rosemary essential oil is also thought to relieve symptoms of dyspepsia and it is an appetite stimulant. Furthermore, research has shown the essential oil to be detoxifying for the liver, and it also helps to regulate the creation and release of bile, which is a key part of the digestive process. It also stimulates blood flow and improves circulation, which can benefit the absorption of nutrients from food. Rosemary leaves are often added to meat dishes because it is particularly helpful in digesting meat, particularly lamb, beef and pork.

2.4. Methods of essential oil production

Essential oils can be extracted using a variety of methods, although some are not commonly used today. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end product. The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as steam distillation, solvent extraction, supercritical fluid extraction, etc.

2.4.1. Steam Distillation

The vast majority of essential oils are produced by steam distillation. There are, however, different processes that are used. In all of them, water is heated to produce steam, which is used to extract the most volatile aromatic chemicals. The steam is then cooled (in a condenser) and the resulting distillate is collected. The essential oils will normally float on top of the hydrosol (the distilled water component) and may be separated off. Steam distillation is the most commonly used method for extracting essential oils. Many traditional distillers favor this method for distilling most oils as they claim that none of the newer methods produces better quality oils (Boucard et al., 2005)

Steam distillation, as described by Boucard et al. (2005), is carried out in a still in which fresh or sometimes dried plant material is placed in a chamber of the still. Pressurized steam, generated in a separate chamber, is then circulated through the plant material. The heat of the steam forces open the tiny intercellular pockets in which the essential oils are contained releasing the oils. During steam distillation, the temperature of the steam must be moderated so that it is high enough to open the oil pouches without destroying the plants, fracturing or burning the essential oils as has been recommended in the literature (Sheridan, 2000). Some or most essential oils have been found to be heat sensitive and hence thermo degradable. As the tiny droplets of essential oils are released, they evaporate and mingle with the steam, travelling through a pipe into a condenser. The steam and oil vapour are then condensed to a liquid mixture.

As the oil-water mixture has been found to be nearly immiscible at a temperature lower than about 65℃ (Sheridan, 2005). The mixture can be separated using various gravity related techniques. Due to the immiscibility of the oil and water at low temperature, the essential oil can be separated from the water by either decanting off the water or skimming of the oil from the top, as the oil is less dense than water at these conditions. The density of some essential oils such as lavender oil has been reported to average 0.89g /L, as opposed to lg/L (Ndou,1986) for water at room temperature and atmospheric pressure conditions. The water obtained as a by product of distillation is referred to as floral water or distillate and retains many of the therapeutic properties of the plant. For this very reason, floral waters are valuable in skin care for making facial mists and toners and are also preferred to essential oils when treating a sensitive individual or child or when a more diluted treatment is required (Sheridan, 2000).

2.4.2. Water/hydro distillation

In this method, the charge (which is usually comminuted) is immersed totally in boiled water. The stills are of the simplest type as shown in Fig 2.1 and are used extensively by smallholder producers of essential oils (Guenther, 1972). Often they are heated over an open fire.

Disadvantages of the Hydro Distillation

- The process is slow and the distillation time is much longer thereby consuming more fuel making process uneconomical.
- Variable rate of distillation due to difficult control of heating.
- Extraction of the herb is not always complete.
- As the bottom walls of the still comes in direct contact with the fire from furnace there is a possibility of adjacent plant material getting charred and thus imparting an objectionable odor to the essential oil.
- Prolonged action of hot water can cause hydrolysis of some constituents of the essential oils such as esters, which reacts with the water at high temperatures to form acids and alcohols.
- Not suitable for large capacity / commercial scale distillations and not suitable for high Boiling hardy roots / woody plant materials (Guether, 1960).

However, it is necessary for the efficient distillation of certain woody materials e.g. sandalwood and cinnamon barks (Noor Azian, 2001).

2.4.3. Steam and Water distillation

To overcome the drawbacks of the water/ hydro distillation, the technique was modified and wet steam distillation was developed. The plant material is supported on a cage / perforated grid below which water is boiled. Direct contact of plant material with hot furnace bottom is thus avoided. The water below the grid is heated by open fire which produces saturated and wet steam which rises through the plant material vaporizing the contained essential oil. Figure 2.1 shows parts of steam/water distillation unit.

Disadvantages:

- Unsafe, time consuming due to low pressure steam, poor quality oil.
- Improper condensation, oil separation incomplete, less recovery.
- Poor material of construction and excessive pollution (Guether, 1960).

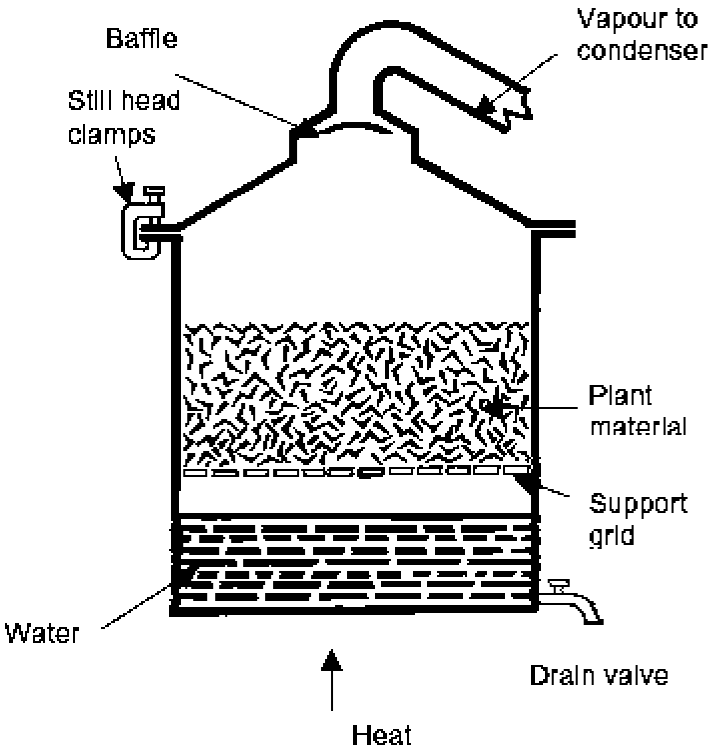


Figure 2.1 Water / steam distillation unit (Guenther, 1972)

2.4.4. Hydro-diffusion

Although introduced more recently than carbon dioxide extraction, hydro-diffusion is similar to steam distillation except that the steam is produced above the plant material and percolates down through it (Chrissie, 1996). The advantage of hydro-diffusion over distillation is that the process is quicker, especially for fibrous material such as woods and barks. The resultant oils are reported to have a superior aroma and a richer colour obtained by ordinary distillation. Nevertheless, oils captured by hydro-diffusion process are not widely available.

2.4.5. Distillation with cohobation

Most essential oils have finite solubility in water but in certain oils like the oil of rose the solubility is quite high. In such a situation the loss of oil taking place with the outgoing waters of distillation can become alarmingly high. This problem is solved by returning the condensed water from the separator back to the extraction chamber. It cannot be done with steam distillation as the water level in the extraction chamber will keep on building up due to continuous steam injection. Instead the distillation is carried out in the mode of water and steam distillation. Condenser is moved above the distillation still so that condensed water from separator can flow by means of gravity to the extraction chamber. By limiting the total quantity of water in this closed cycle operation, it is possible to obtain increased yields of essential oils which are more water soluble. Cohobation of distillation waters in separate cohobation towers is quite widespread in Soviet Union and Bulgaria (Guenther, 1960).

2.4.6. Solvent Extraction

The purpose of distillation is to separate a mixture of several ingredients by taking advantage of their different volatility, or to separate volatile ingredients of a raw material from its non-volatile parts. If the final product is too sensitive to heat or humidity, solvent extraction could be used. Solvent extraction is adapted in producing essential oils generated by some flowers (Rose, Violetta, and Geranium), gums and resins. The raw material is placed in a glass, vessel and soaked with a suitable solvent (petroleum, ether or benzene). After the extraction, the solids are separated from the liquid mixture. The latter is heated so that the more volatile essential oils can be evaporated to be subsequently condensed. Alternatively if the solvent is more volatile, such as ethanol, it could then be vaporized leaving behind the essential oils (Ndou, 1986). As solvent extraction uses very little heat, it is found to be advantageous in producing essential oils with whole fragrances that would otherwise be destroyed or altered during steam distillation. Therefore, this extraction technique can be used to extract essential oils from very delicate plants to produce higher amounts of essential oils at lower costs (Ndou, 1986). There are, however, some disadvantages associated with the solvent extraction technique. Solvent residues often contaminate the product causing side effects which make the use of essential oil undesirable for skin applications but could still be fine for fragrances or perfumes (Ndou, 1986). Therefore, with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the essential oils.

This process also sometimes yields an aromatic resinous product known as oleoresin, which is more concentrated than essential oils with an even wider application in the food and other industries (Heath, 1981).

2.4.7. Supercritical fluid extraction

Supercritical fluid extraction (SFE) of essential oils is a modern technique, currently being applied in the process industry, which competes with conventional processes such as steam distillation and organic liquid (solvent) extraction. It has been widely accepted by many investigators that SFE provides a rapid and quantitative method for extracting essential oils from aromatic plants that compares favorably with steam distillation (Kerrola, 1995). A single-component fluid is said to be supercritical when its temperature and pressure both exceed their critical values, without being far from the critical state (Gaspar et al., 2002). At these elevated conditions the properties of the fluid has both liquid and gas properties.

All materials have a critical point, but for some materials, however this state is more easily reached than others. Carbon dioxide is the most commonly used fluid in extracting essential oils and its application and technique have been extensively researched. Supercritical extraction by carbon dioxide is isolation and separation process taking advantage of the fact that above critical conditions of 31.1℃ and 78.8bar, carbon dioxide cannot be liquefied by any further increases in pressure. Whilst the carbon dioxide is in this supercritical state, the dense gas gains a considerable solvent power, dissolving the primary target such as the essential oil in the plant material. The pressure is then dropped in the separator, causing the carbon dioxide to lose its solvating power and hence releasing the extracted oil drops, leaving behind a high purity essential oil extract. This high purity of these products has recently attracted the attention of the pharmaceutical industry, due to the heavily regulated trade demands for lower quantities of solvent in the final product. This area of research is currently being pursued and analyzed by various researchers such as (Gaspar et al., 2002), in their work for various pharmaceutical companies worldwide, in developing new production techniques based on supercritical carbon dioxide. Using carbon dioxide is especially useful as it is cheap, clean and intrinsically safe with no harmful residues like solvents in the extracts that would be found for solvent extraction as discussed before. The operating temperatures are relatively low, which enables thermally labile compounds to be extracted. This method of extraction has also been reported to ecologically harmless in literature (Gaspar et al., 2002) as no harmful residues of toxic solvents result from it.

supercritical fluid extraction by carbon dioxide has therefore been described by (Simon, 1990) as a new method; potentially commercially viable but is less common and beyond the financial means of most small scale processors. Fig 2.2 shows the flow diagram of supercritical fluid extraction process.

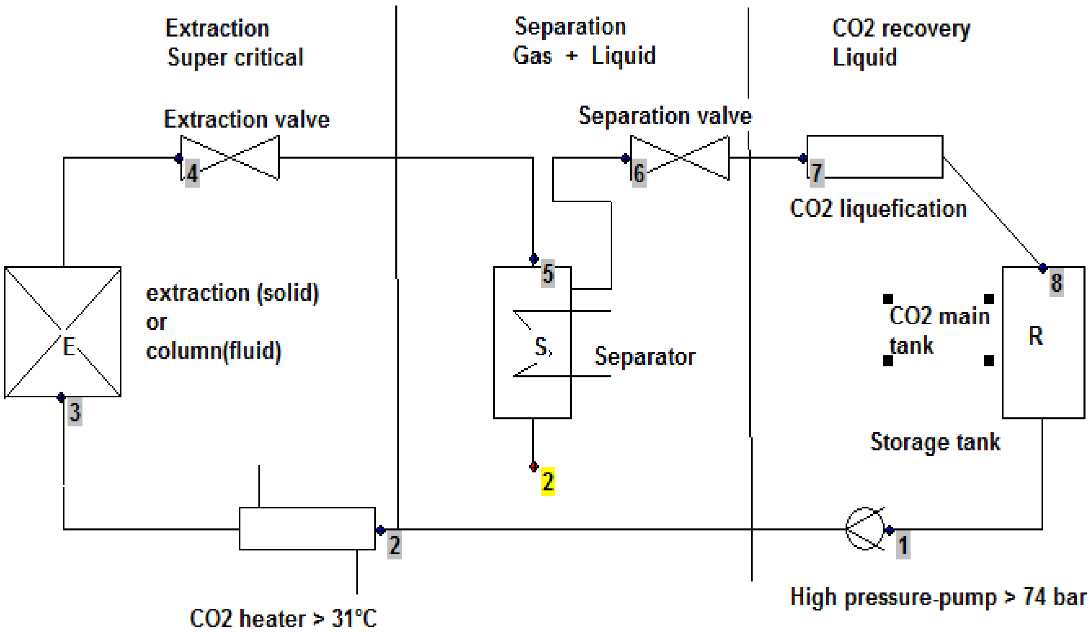


Figure 2.2 Supercritical fluid extraction process (Kerrola, 1995).

2.4.8. Cold pressed expression method

Another method of extraction essential oils as described in the literature (Stable et al., 1988) is cold pressed expression, or scarification. It has been reported to be used mainly to obtain citrus fruit oils such as bergamot, grapefruit, lemon, lime, mandarin, orange, and tangerine oils (Wu et al., 2001). In this process, the fruit is rolled over a through with sharp projections that penetrate their peels thereby piercing the tiny pouches containing the essential oil. The fruit is then pressed to squeeze out the juice from the pulp thereby releasing the essential oils from the pouches. The essential oils rise to the surface of the juice and are separated by centrifugation.

As discussed in the literature (Stahl et al., 1988), cold pressing is more competitive for specific raw material than methods such as supercritical fluid extraction as it is extremely fast, cheap and does not pollute the extracts, although it does not provide a way of selectively extracting essential oils. Figure 2.3 shows cold pressed expression machine.

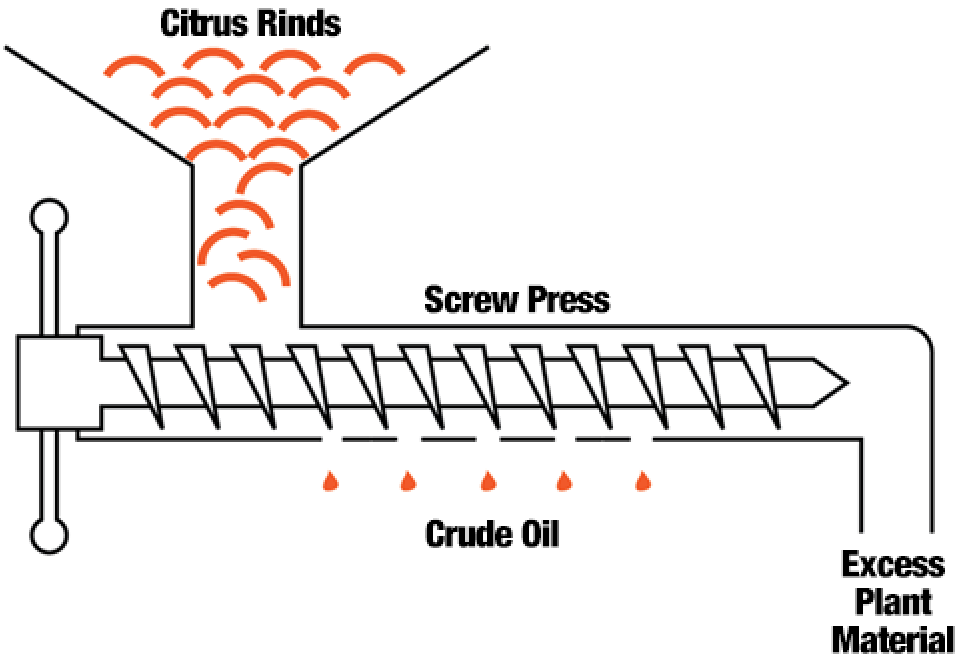


Figure 2.3 Cold pressed expression machine

2.4.9. Effleurage method of extraction

Some flowers, such as jasmine or tuberose, have such low contents of essential oils and are so delicate that heating them would destroy their blossoms before releasing the essential oils. In such cases, an expensive and lengthy process called effleurage is sometimes used to extract the essential oils. As described in the literature (Stahl et al., 1988), flower petals are placed on trays of odorless vegetable or animal fat, which absorb the essential oils from them. At the end of every day or even after a few hours, when the vegetable or fat has removed as much of the essential oil as possible, the depleted petals are removed and replaced with fresh ones. This procedure is repeated until the fat or oil becomes saturated with the essential oil. Adding alcohol to this effleurage mixture separates the essential oils. This method employs a similar operating principle and technique to what was discussed for solvent extraction**.**

2.4.10. Microwave extraction

Microwave energy is a superior alternative to several thermal applications owing to its efficient volumetric heat production. The volumetric heating or heating of the bulk as opposed to transferring heat from the surface, inwards, is more efficient, uniform and less prone to overkill or supererogation. Controllability is by far the greatest advantage of microwaves over conventional thermal technologies. In processing applications, the ability to instantaneously shut the heat source makes enormous difference to the product quality and hence the production economics. The raw material is heated directly by microwaves and this brings about quality consistency and minimizes the impact on the environment as opposed to using fossil fuels or less efficient, indirect electrical heating systems. Specifically in the essential oil extraction, microwave mediated processes are highly desirable due to their small equipment size (portability) and controllability through mild increments of heating. However, so far the microwave technology has found application in very few industrial bio-processing installations due to the lack of available data on microwave interaction with heterogeneous natural raw materials. The sensing and close control of microwave process is a challenging science and there seems to be insufficient literature in this regard.

2.4.11. Vapor-Cracking

This is a new continuous process, which is French patented (Martel, 1978), allows the recovery of the most volatile aromas directly from solids, like fruits, vegetables and other aromatic raw materials. As a first step in processing, either batch wise or continuously, it simultaneously replaces a press, particularly when that conventional process would yield very little juice. Heating is obtained by direct steam injection by steam jacket and hollow screws, or by any suitable range of radiations. Condensates may be recovered from that “bleaching” if some preliminary extraction has occurred. A flash chamber is kept under vacuum, where the pre-heated solids fall after passing a sluice. At this point, 10% of the water content of the material is vaporized. The recovered condensates are remarkably rich in the most volatile aromatic fractions. Continuous distillation or any other suitable process further concentrates them.

2.4.12. Turbo-Extractor

This method is used for solvent or water-extraction of numerous solid raw materials, mainly vegetal, used in the perfume, flavour, cosmetic, pharmaceutical, spirit and food industries. It is energy and time saving production of natural extracts. The equipment is equipped with a high blade turbine. It is used for crushing materials within the solvent and fluidizing the comminuted pieces. Extraction is accelerated by the intimate and turbulent contacts between the solid and the liquid phase. Nevertheless, this type of equipment is very expensive and not economical.

2.5. Important physical and chemical properties of essential oils

The chemical properties of essential oils depend on the natural factors such as type of species, the geographical origin and location of the plant, time of harvesting, plant parts from which the oils are extracted (Dey, 1996). Essential oils components and percentage are different from oil to oil even for the same botanic plant due to:

- **Weather and planting time**

Most of herbs are planted but small amounts could also be wild grown or collected plants. By means of an example with spearmint, the oil percentage from a summer crop is double that from a winter crop. The oil percentage from a given summer could be different from a previous summer even from the same field. The component analysis of the oil could also be different from one season to another.

- **Soil elements**

The B-phellanderene percentage increases in marjoram oil with the higher levels of molybdenum manganese, copper, calcium, zinc or iron in the soil.

- **Irrigation**

The highest yield of plant material results from increasing the leaf area. For example, this will happen if a basil field is irrigated every four days. The essential oil is highest at medium levels of soil moisture.

- **Time of harvest**

The peppermint oil yield increases as the herb approaches maturity in the full bloom stage.

- **Length of distillation operation**

To specify time for distillation operation you must consider whether the herbs (species) are fresh, faded, or dry. It would take additional time for distillation if the herb is faded than if it is fresh. Leaves take less time than seeds because leaves are thinner than seeds and cells are more concentrated in leaves than seeds (Guenther, 1960).

2.5.1. Physical properties of essential oils

2.5.1.1. Specific gravity

Specific gravity is an important criterion of the quality and purity of an essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at 15℃. In general, the specific gravity is less than 1.0 (Guenther, 1960). Hence essential oil can be collected over (floating on) water.

2.5.1.2. Optical rotation

Most essential oils when placed in a beam of polarized light possess the property of rotating them plane of polarization to the right (dextrorotatory), or to the left (laevorotatory).The degree of rotation and the direction are important indicators of purity.

2.5.1.3. Refractive index

When a ray of light passes from a less dense to a more dense medium, it is bent or "refracted" toward the normal. If *e* represents the angle of refraction and *i* the angle of incidence, according to the law of refraction,

$$\frac{\sin \mathrm{I}}{\sin e} = \frac{N}{n} \quad \text{……………………………………………………………......................} \quad (2.1)$$

Where *n* is the index of refraction of the less dense, and N is the index of refraction of the denser medium. Refractometers offer a rapid and convenient method for the determination of this physical constant.

2.5.1.4. Molecular refraction

The index of refraction of a liquid varies with temperature and the wave length of the light. In order to compare the refractivity’s of different liquids, the use of molecular refractivity (molecular refraction) is necessary.

2.5.1.5. Solubility

- **Solubility in Alcohol**

Most essential oils are only slightly soluble in water and are miscible with absolute alcohol. The solubility of oil may change with age.

- **Solubility in water**

Most of essential oils of commercial interest are steam volatile, reasonably stable to action of heat and practically insoluble in water and hence suitable for processing by steam distillation.

2.5.1.6. Boiling range

In the case of isolates and synthetics, the boiling range is an important criterion of purity.

2.5.1.7.Evaporation residue

An important criterion of purity is the evaporation residue; i.e., the percentage of the oil which is not volatile at 100℃. It is important to study the odor of oil as it volatilizes during the heating.

2.5.1.8. Flash point

The flash point may prove useful in the valuation of an essential oil. The flash point has value as an indication of adulteration: additions of adulterants such as alcohol and low boiling mineral spirits will greatly lower the flash point.

2.5.2. Chemical properties of essential oils

In general, essential oils consist of chemical compounds that have hydrogen, carbon, and oxygen as their building blocks. These can be subdivided into two groups: the hydrocarbons, which are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes, and diterpenes); and the oxygenated compound (mainly esters, aldehydes, ketones, alcohols, phenols) and oxides; acids, lactones, sulphur and nitrogen compounds are sometimes also present.

2.5.2.1. Aldehydes

Citral, citronellal, and neural are important aldehydes found notably in lemonscented oils such as Melissa, lemongrass, lemon verbena, citronella, etc. Aldehydes in general have a sedative effect; *citral* has antiseptic properties.

2.5.2.2. Phenols

These tend to have a bactericidal and strongly stimulating effect, but can be skin irritants. Common phenols include *eugenol* (found in clove and West India bay), *thymol* (found in thyme), *carvacrol* (found in oregano and savoury).

2.5.2.3. Terpenes

Common terpene hydrocarbons include limonene (antiviral, found in 90% of citrus oils) and pinene (antiseptic, found in high proportions in pine and turpentine oils). Sesquiterpenes have outstanding anti- inflammatory and bactericidal properties.

2.5.2.4. Ketones

Some of the most common toxic constituents are ketones, such as thujone found in *mugwort*, tansy, sage and wormwood; and *pulegone* found in pennyroyal and *buchu*. Non-toxic ketones include jasmine (in Jasmine) and *fenchone* (in fennel oil).

2.5.2.5. Oxides

By far the most important oxide is cineol (or eucalyptol). It has an expectorant effect, and is well known as the principal constituent of eucalyptus oil. It is also found in a wide range of other oils, especially those of a *camphoraceous* nature such as rosemary, bay laurel, tea tree, and cajuput.

2.5.2.6. Esters

Probably the most widespread group found in essential oils, which includes linalyl acetate (found in bergamot, clary sage, and lavender) and *geranyl* acetate (found in sweet marjoram). They are characteristically fungicidal and sedative, often having a fruity aroma.

2.5.2.7. Alcohols

These compounds have good antiseptic and antiviral properties with an uplifting quality; they are also generally non-toxic. Among the most common terpene alcohols are *linalool* (in rosewood, *linaloe*, and lavender), *citronellol* (in rose, lemon, eucalyptus and geranium) and *geraniol* (found in palmarosa); also *borneol, methol, terpineol, nerol, farnesol, vetiverol,* benzyl alcohol, and *cedrol* (Lawless, 2002)

2.6. Factors affecting yield and quality of rosemary essential oils

The yield and quality of rosemary essential oils have been known to vary due to a number of factors. Such as:

- **Mode of distillation**

Technique for the distillation should be chosen on basis of oil boiling point and nature of herb as the heat content and temperature of steam can alter the distillation characteristics.

- **Proper design of equipments**

Improper designing of tank, condenser, or separators can lead to loss of oils and high capital investments.

- **Material of construction of equipments**

Essential oils which are corrosive in nature should be preferably distilled in stills made of resistant materials like Aluminum, Copper or Stainless Steel.

- **Nature of raw material**

Nature of raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials have to be crushed, dried, powdered or soaked in water to expose their oil cells.

- **Filling of raw material / steam distribution**

Improper loading of the herb may result in steam channeling causing incomplete distillation. Operating parameters like steam injection rate, inlet pressure, condensate temperature. Proper control of injection rates and pressure in boiler-operated units is necessary to optimize the temperature of extraction and for maximal yield. Temperature of condensate should not be too high as this can result in oil loss due to evaporation.

- **Distillation time**

Different constituents of the essential oil get distilled in the order of their boiling points. Thus, the highest boiling fractions will be last to come over when, generally, very little oil is distilling. If the distillation is terminated too soon, the high boiling constituents will be lost.

- **Pre-condition of tank / equipments**

Tanks should be well steamed for multiple crop distillation. Tank / equipments should not be rusted for quality oil (Dey,1998).

2.7. Principle of steam extraction of rosemary essential oil

2.7.1. Preamble

Most essential oils are obtained from plant material by a process known as steam distillation. Steam distillation is based on the assumption that essential oils are volatile in steam and generally insoluble in water.

The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled (and subsequently recovered). In the presence of steam, these substances are volatilized at a temperature close to 100 °C at atmospheric pressure. The mixture of hot vapors will, if allowed to pass through a cooling system, condense to form a liquid in which the oil and water comprise two distinct layers. Most (but not all) essential oils are lighter than water and form the top layer. The steam that is used for the distillation is generated either within the vessel that contains the plant material (by boiling water contained at the base) or by an external boiler.

The use of steam generated within the vessel requires that the plant material be supported above the boiling water by a grid. The water is heated either directly using a burning fuel or by heat exchanger coils. The simplicity of the method makes it suitable for small-scale industrial production. If steam is generated by an external boiler, it is introduced into the base of the vessel via an open coil, jets or similar device(s). The advantages of this type of generation are that it is relatively rapid and allows greater control by the operator. The vessel can be emptied and recharged quickly. With the immediate reintroduction of steam, there is no unnecessary delay in the commencement of the distillation process.

2.7.2. Components of the Extraction Process

Steam distillation process for the extraction of essential oils from plant materials consists of four basic parts:

Steam Generator or Boiler

The dictionary defines steam as "the gas or vapour into which liquid water is changed by boiling, especially when used under pressure as a source of energy".

The primary-objective of a boiler operation is to provide a continuous supply of steam at whatever pressure and temperature are suitable for the end use. The secondary objective is to provide such steam at the lowest possible cost. These objectives translate into "operational efficiency".

There are many boilers available for steam generation. Some are high pressure boilers and other medium to low pressure boilers. Most process steam in industries falls under medium / low pressure. For the purpose of essential oil production, low pressure steam is generally used. The capacity of the boiler will depend on the size of distillation units (James et al., 1926).

Extraction Chamber

The extraction chamber is simply a fixed bed where the hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. The temperature of the steam needs to be carefully controlled at a temperature just high enough to force the plant material to let go of the essential oil, yet not too high as to burn the plant material or decompose the essential oil. The steam, which then contains the essential oil, is passed through a cooling system to condense the steam, which forms a liquid from which the essential oil and water are then separated. The steam is produced at slightly greater pressure than the atmosphere and therefore boils at above 100 °C, which facilitates the removal of the essential oil from the plant material at a faster rate and in so doing prevents damage to the oil (James et al., 1926).

Heat Exchanger

Heat exchanger is a device that provides the flow of thermal energy between two or more fluids at different temperatures. The vapors from extraction chamber are cooled by passing them down a tube that is immersed in a flow of water. The condensate runs by gravity into a container in which the oil and water separate due to their differing specific gravities: the "Separator".

Heat exchangers used in a wide variety of applications. These include power production; process, chemical and food industries; electronics; environmental engineering; waste heat recovery; manufacturing industry; and air-conditioning, refrigeration, and space applications. Heat exchangers can be classified according to the following main criteria:

a. Recuperators / regenerators
b. Transfer processes: direct contact and indirect contact
c. Geometry of construction: tubes, plate and extended surfaces
d. Heat transfer mechanisms: simple-phase and two-phase
e. Flow arrangement**:** parallel, counter, and cross flows

The major construction types are tubular, plate and extended surface heat exchangers. Tubular heat exchangers are built of cylindrical tubes. One fluid flows inside the tubes and the other flows on the outside of the tubes. Tube diameter, the number of tube length, the pitch of the tubes, and the tube arrangement can be changed. Therefore, there is considerable flexibility in their design.

Tubular heat exchangers can be further classified as follows:

- Double-pipe heat exchangers
- Shell-and-tube heat exchangers
- Spiral tube heat exchangers

Shell- and tube heat exchangers are built of tubes mounted in large cylindrical shells with the tube axis parallel to that of the shell. They are widely used as oil coolers, power plant, steam generators in nuclear power plants, in process applications, and in chemical industry. One fluid stream flows through the tubes while the other flows on the shell side, across or along the tubes. In a baffled shell-and-tube heat exchanger, the shell-side stream flows across between pairs of baffles and then flows parallel to the tubes as it flows from one baffle compartment to the next. There are wide differences between shell-and-tube heat exchangers depending on the application. The main design objective here is to accommodate thermal expansion, to furnish ease of cleaning, or to provide the least expensive construction if other features are of no importance. In a shell-and-tube heat exchanger with fixed tube sheets, the shell is welded to the tube sheets and there is no access to the outside of the tube bundle for cleaning. This low-cost option has only limited thermal expansion, which can be somewhat increased by expansion bellows. Cleaning of the tube is easy. A number of shell-side and tube-side flow arrangements are used in shell-and-tube heat exchangers depending on heat duty, pressure drop, pressure level, fouling, manufacturing techniques, cost, corrosion control, and cleaning problems. The baffles are used in shell-and-tube heat exchangers to promote a better heat transfer coefficient on the shell-side and to support the tubes (James et al., 1926).

Separator

The mixture of condensed oil and water runs into the separator where the lighter insoluble oil floats on the surface and accumulates slowly and from where it is drawn off periodically. This is the essential oil. The water, or rather water containing the more water soluble constituents extracted by the steam, known as the hydrolat or "floral water" is drawn off continuously from the bottom of the separator. Or, in separating the water from the oil, the water layer was carefully run out from the bottom of the decanter by opening the tap until its meniscus is just at the calibration mark. The contents that remained inside the decanter which were now the oil layer and the water between the tap bridge and the bottom of the calibration mark were weighed on the electronic analytical balance. The isolation of the water from the essential oil has to be done as carefully and accurately as possible in order to minimize the effect of human error due to any inaccuracies in leveling the water and oil layer to coincide with the level calibration mark (James et al., 1926).

2.7.3. Basic scientific principles involved in steam distillation process

In steam extraction to convert, any liquid into a vapour we have to apply energy in the form of heat called the latent heat of vaporization. A liquid always boils at the temperature at which its vapour pressure equals the atmospheric / surrounding pressure. For two immiscible liquids, the total vapour pressure of the mixture is always equal to the sum of their partial pressures. The composition of the gas mixture will be determined by the concentration of the individual liquid components and their respective partial pressures. As known, the boiling point of most essential oil components exceeds that of water and generally lays 150-300℃.

Consider a sample of an essential oil having a component 'A' with a boiling point of 190℃ and water having a boiling point of 100℃; when these two immiscible liquids are brought together, and once their vapours have reached saturation, the temperature will immediately drop to 99.5℃, which is the temperature at which the sum of the two vapour pressure equals 760mm Hg.

$$\mathrm{P_{total} = P_A + P_{wate}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

Where:

$\mathrm{P_{total}}$: is total pressure of the mixture

$\mathrm{P_A}$: is partial pressure of component A

$\mathrm{P_{water}}$: is partial pressure of water

Thus any essential oil having high boiling point range can be evaporated with steam in a ratio such that their combined vapour pressures will be equal to the atmospheric pressure and can be isolated from the plants by the wet distillation process (Guenther, 1960).

2.7.4. Treatment of the plant material

The chief application of distillation is the initial isolation of essential oils from the aromatic plant material, that is, comminution of the plant material. This process involves the handling of predominantly solid products, and the preparation of the material must therefore, be carried through carefully if the most efficient and complete recovery of the valuable essential oils is to be assumed. The essential oils are enclosed in “oil glands” veins, oil sacks or glandular hairs of the aromatic plants. If the plant material were left intact, the oil could be removed (vaporized) by steam only after they had passed through the plant tissue to an exposed surface. The process, which can be accompanied by hydro-diffusion. Diffusion is a very slow process, and if the plant were left intact, the rate of recover y of oil would be determined entirely by the rate of diffusion. Consequently, before distillation, the plant material must be disintegrated to some extent. This disintegration process, commonly termed comminution results in exposing directly as many oil glands as is practically possible. It always reduced the thickness of material through which diffusion must occur; greatly increase the rate of vaporization and distillation of essential oils. All actually exposed oil will be entrained by passing steam and carried away from the plant. Extent of comminution required varies with the nature of the plant material: flower, leaves and other thin and non-fibrous parts of the plant can be distilled without comminution. The cell walls in these plants are in most cases sufficiently thin and permeable to permit rapid removal of the oil. Seed (fruits) on the other hand, must be thoroughly crushed in order to expose a greater number of oil glands.

It should be noted that the principal purpose of comminution is to render the essential oil readily removable by the passing steam. It is evident that once the plant materials has been crushed or reduced in size, it must be distilled immediately. Otherwise, the essential being volatile will partly evaporate, with two adverse effects. Firstly the total yield of the oil be reduced and secondly the composition of the oil will change. Thereby, affecting the odour of the oil since essential oils are mixture of several compounds, the more volatile compounds evaporate to a greater extent than the higher boiling and less volatile ones. The storage of the plant materials before comminution also offers some hazards in the way of ultimate loss of volatile oil. Changes in the physiochemical oils distilled either from fresh or from dried plants show wide variations in physiochemical properties and chemical composition. With many oils, it is advisable, therefore, to state whether they were distilled from fresh, wilted or air-dried plant material. This is especially true of flower, leaves, herbs and roots which in fresh state contain much moisture content**.**

2.7.5. Treatment of the volatile oil, distillation water and spent plant material

The quality of the condensed oil may deteriorate, particularly if oil must be stored for some time just as the condensed water (distillation water) is always saturated with oil, so the condensed oil will always be saturated with water. There is also probability of slow reaction between oil and water, unless the latter is almost completely removed the oil can be brightened (clearly of cloudy appearance) by filtering through kieselgular or magnesium carbonate on filter paper. This procedure removes small droplet of water which causes the cloudiness, but does not completely dry the oil. Large quantity of the oil may be filtered through mechanical filters, filter press, or run through high-speed centrifuges. The distillation water flowing off the oil separator contains some of the volatile oil in solution or suspension, the quantity depending on the solubility and specific gravity of the various oil constituent. Such distillation water cannot be discarded but must be subjected to further treatment to prevent loss of oil. In case of water distillation, it may automatically be returned into the retort during distillation.

The disposal of the spent plant material which represent a large bulk offers an annoying problems. One economical method of disposal consists in using it as fuel after air drying, either in sun or near the still in case of direct fire stills. Since the used or spent material has a low fuel value per unit volume consideration must be given to the construction of a special fuel box. In most cases, the spent material may be used effectively as fertilizer. Certain spent materials make the excellent cattle feed, this is particularly true of seeds which contain a high percentage of protein and fatty oil. The drying is done in a dehydrating apparatus or by air drying on shelves. When sweetened with molasses, some spent grasses such as lemon grass seemed to be relished by cattle.

3. Material and Methods

3.1. Materials

Raw materials:

Rosemary leaves (*Rosmarinus officinalis*)

Equipements:

- ✓ Steam generator Boiler
- ✓ Extraction chamber
- ✓ Heat exchanger
- ✓ Separator
- ✓ Gas chromatography - Mass spectroscopy
- ✓ UV visible spectroscope
- ✓ Electric heater
- ✓ Centrifugal separator
- ✓ Digital weighting balance,
- ✓ Water bath
- ✓ Separation funnel,
- ✓ 250ml and 1000ml Beakers
- ✓ 2000 ml Round bottom flask
- ✓ vibro-viscometer ,
- ✓ pH meter,Oven, sieve, density separator, density bottle (pycnometer) Obscured glass bottles

Chemicals:

- Distilled water, Ethanol, Anhydrous sodium sulfate, sodium bisulphate, glycerin, sodium hydroxide, potassium hydroxide, oxalic acid, phenol phathaline indicator,

3.2. Methodology

3.2.1. Collection of plant material

A bulk sample of Rosemary leaves was collected from a single location from Sebeta Region which is a town and separate Wereda in centeral Ethiopia, on February 2016 at morning time. Sebeta is located in the Oromia Special Zone Surrounding Finfinne of the Oromia Region, this town has a latitude and longitude of $8^{\circ}55^{'}\mathrm{N}$ $38^{\circ}37^{'}\mathrm{E}$ / $8.917^{\circ}\mathrm{N}$ $38.617^{\circ}\mathrm{E}$ coordinates and having an elevation of 2356 m above sea level. (Wikipedia, the free encyclopedia, 2016)

The dried plant material was taken to the laboratory of the Botanic Department in Addis Ababa University, Colleague of Science Faculty, to determine the species of the plant material. After that the bulk material were divided in to three equal parts. The first group were dried in the day sun light, the second group were dried at shade place without exposing for sun light and the third and last group were put as fresh.

3.2.2. Treatment of the rosemary leaves

A sample with a mass of approximately 40 kg was prepared. One full sunny day drying period was implemented for the first group and two and half day for the second groups (Kasumba, 2003). After completion of drying, the leaves were re-weighed to determine any potential mass loss as a result of the evaporation of both moisture and volatile oil components due to the drying effect.

3.2.3. Size reduction

After the moisture was removed by sun drying and air drying rosemary leaves were cut with a scissors for size of around 2 cm in order to increase contact area of the leaves.

3.2.4. Determination of moisture content of the rosemary leaves

Five samples of the rosemary leaves were randomly selected from each group ,weighted and dried in oven at 105℃ and the weight was measured every two hours. The procedure was repeated until a constant weight was obtained and the percentage moisture content of the rosemary leaves was determined (General Health Welfare, & Delhi, 2005).

$$\text{Moisture content } \% = \frac{W_1 - W_2}{W_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Where:

W_1= original weight of the sample before drying

W_2= weight of the sample after drying

3.3. Essential oil extraction

Figure 3.1 photograph of steam distillation equipment

The extraction was carried out at different drying condition and extraction time by maintaining extraction temperature of 94℃ - 95℃ to avoid thermal degradation of the essential oil components. Moreover, the pressure was set at the atmospheric pressure. The rosemary leaves size is also maintained constant at 2cm. Figure 3.1 shows The set-up of steam distillation equipment. It consists of distillation stills, boiler and condenser. The distillation still has a capacity of 6kg plant material per batch. However, in this research one kilogram of rosemary leaves were tested for each experiment. Three different drying conditions such as sun dried rosemary leaves, shade dried rosemary leaves and fresh rosemary leaves were tested for four different durations of 30 min, 60 min, 120 min and 150 min, to compare their efficiency based on the oil yield and the oil composition. This time limit was made by conducting preliminary test on rosemary leaves using steam distillation.

The oil extraction was conducted using batch steam distillation equipment, which essentially consisted of a static bed. This batch equipment possesses have advantages such as, ease of operation, simple construction and relatively low capital construction cost. The experimental equipment (steam distillation) consists of the following major components.

Boiler*:* The boiler connected to three-phase power, consisted of a pressurized air tight stainless steel container and could contain up to 24 liter of the water. This was used to generate steam for stripping the plant material. It absorbed make up water from the running tap water automatically.

Extraction chamber: This served primary as a container for the plant material, and as a vessel in which the steam contacts the plant material and vaporized its essential oils. It consisted of a cylindrical container manufactured in stainless steel with a diameter of 60cm and 70cm high, was equipped with a removable cover which could be clamped upon the cylindrical section. On the top of the cylindrical section, a pipe was attached to lead the vapors to the condenser. The bottom of the extraction chamber was provided with a drain valve sufficiently wide so that any water condensing within the charge and dripping to the bottom could be drawn off in the course of distillation. Figure 3.2 shows the photograph of extraction chamber.

Figure 3.2 photograph of extraction chamber equipment

Condenser: A shell and tube condenser was used to convert all of the steam and the accompanying oil vapors from the top of the extraction chamber into liquid. As shown in figure 3.3, water was fed to the overhead and this permitted the water to trickle over the entire length of the condenser tubes. It will be noted that the condenser tubes were all sloped downward slightly, to insure proper drainage of the condenser oil and steam. The cooling medium used in this device was cooling water drawn from a running tap.

Figure 3.3 photograph of Shell and tube Condenser.

Separator: the condensed mixture of oil and water was collected in the separator. The whole apparatus was properly sealed to avoid leakage. Oil being lighter than water floats on the surface of water below 65℃ (Sheridan, 2005). Hence, the mixture of oil and water were separated in the separating funnel.

Anhydrous sodium sulphate was used for dehydrating the water that was mixed with the oil. The products were weighted and physicochemical properties of essential oil were determined.

3.4. Characterization of the extracted rosemary essential oil

The yield of essential oil, specific gravity at 25℃, density, dynamic viscosity, kinematic viscosity ,pH value ,flash point, refractive index, optical rotation ,solubility in ethanol were analyzed using standard procedures. The acid value, saponification value, iodine value and total aldehyde content of rosemary essential oil were performed and compared with literatures.

3.4.1. Determination of physical properties

3.4.1.1. Percentage of rosemary essential oil extracted

The percentage yield of rosemary essential oil was calculated using two formulas, i.e. percentage of oil yield and percentage of extraction yield.

$$\text{Percentage of oil yield} = \frac{\text{mass of oil}}{\text{mass of oil percent in the rosemary}} * 100\% \ldots\ldots (3.2)$$

Since rosemary have an oil content of 1-1.5%, average 1.25% oil content was taken for calculating the yield, Therefore:

$$\text{Percentage of oil yield} = \frac{\text{mass of the oil}}{1.25\%\ \text{mass of the sample}} * 100\% \ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

The second form that the yield calculated was

$$\text{Percentage extraction yield} = \frac{\text{mass of oil}}{\text{mass of the sample}} * 100\% \ldots\ldots\ldots\ldots\ldots (3.4)$$

Here in this research work the second method of yield calculation is used.

3.4.1.2. Specific gravity of essential oil

The density of the oil was determined by using density bottle. A clean and dry empty bottle of 25ml capacity was weighed (W_0) and then the bottle was filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2) (General Health Welfare & Delhi, 2005). The expression for specific gravity (Sp.gr) is:

$$\text{Sp. gr.} = \frac{W_1 - w_0}{W_2 - w_0} = \frac{\text{mass of sample}}{\text{mass of an equal volume of water}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

3.4.1.3. Kinematic viscosity of essential oil

Kinematic viscosity of the essential oil was measured indirectly using vibro-viscometer which is available in laboratory of school of chemical and bio engineering. Initially, a sample was heated at a temperature of 20°C. A sample of 35ml oil was measured and fed to a sample holder of the vibro-viscometer. A sensor of the viscometer was immersed to the oil and then a dynamic viscosity of oil was displayed on the vibro-viscometer screen at a temperature of 20°C. Then the kinematic viscometer was calculated.

$$\text{Kinematic viscosity} = \frac{\text{Dynamic viscosity of the sample}}{\text{Density of the sample}} \ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

3.4.1.4. pH value of essential oil

2g of the sample was taken and putted in a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. Then it was cooled in cold-water bath to 25℃. First, the pH electrode was standardized with a buffer solution and then immersed in the sample and the pH was read (A.O.A.C, 2000).

3.4.1.5. Boiling Point of essential oil

25 ml of rosemary essential oil poured in to beaker and a thermometer was inserted and placed on a heating mantle, it was observed that the oil in the beaker started circulating leading to boiling of oil and read temperature on thermometer.

3.4.1.6. Refractive index of essential oil

The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. For practical measurements, including this method, the scales of standard instruments indicate refractive indices with respect to air rather than vacuum. Refractive Index of the sample was determined indirect method using spectrometer (He-Ne Leather). It was ascertained that the temperature of the spectrometer is 25.0℃ at room temperature, wave number 632.8nm and the prism was clean and completely dry. The prism with sample was placed in the cuvet and adjusted the instrument and light to obtain the most distinct reading and then determined the refractive index using Brewster's angle. Brewster's angle formula was used to calculate refractive indices of the interface.

$$\mathrm{N} = \tan(\theta_b) \qquad (3.7)$$

Where:

N =refractive index,

θ_b = Angle of refraction, the refractive index of air is 1.

The index of refraction of oils is characteristic within certain limits for each kind of oil. It is related to the degree of saturation particularly to the extent of conjugation, but it is affected by other factors such as free fatty acid content, oxidation, and heat treatment.

3.4.1.7. Moisture and volatile matter of rosemary essential oil

4gm of oil was weighted and putted in a dish and then dried in an oven at 105℃ for 1h. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed and the moisture and volatile matter of the oil was determined (Hand book of food analysis, 1984).

3.4.1.8. Optical rotation of essential oil

Polari meter with a standard 10 mm tube (cuvet) and a sodium vapor lamp was adjusted and volatile oil sample and instrument temperature was 25℃ (98°F).The Polari meter tube was filled with oil and wipe off excess oil on the exterior. Then the tube is placed in the Polari meter and the analyzer is slowly turned until both halves of the field were viewed through the telescope, reading was observed to clockwise (+) and anti-clock wise (-) (General et al., 2005).

3.4.2. Determination of the chemical properties of rosemary essential oil

3.4.2.1. Acid value

Acid value is the mass of potassium hydroxide (KOH) in mg that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound. The acid number is used to quantify the amount of acid present, in rosemary essential oil sample. Acid value was determined according to the method of European Pharmacopeia.

Rosemary essential oil (0.5g) was accurately weighted and dissolved in 10ml of 95% ethanol and 2-3 drops of phenolphthalein indicator was added. The free acid was then titrated with standard 0.1 N aqueous sodium hydroxide solutions by adding the alkali drop-wise at a uniform rate of about 30 drops per minute. The content of the flask was continuously agitated. The primary manifestation of the red coloration that did not fade within 10 seconds was considered the end point. Afterward, the acid value was determined using the following equations:

$$\text{Acid value} = \frac{5.61 * (\text{number of ml of } 0.1\text{N NaOH})}{\text{Weight of sample in gram}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3.8)}$$

3.4.2.2. Saponification Value

Saponification value represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1g essential oil under the condition specified. Saponification value was calculated by European Pharmacopeia standard procedure.

Rosemary essential oil (0.5g) was accurately weighed and dissolved in 10ml of 70% ethanol and then 10ml of 2.5N KOH solution was added. This procedure was performed together with blank experiment which was also performed omitting the oil. The mixture was refluxed for two hours then cooled. The unreacted KOH was titrated with standard N/2 oxalic acid by adding 2-3 drops of phenolphthalein indicator. After that, the saponification value was determined using the following equation (International Letters of Chemistry, Physics and Astronomy).

$$\text{Saponification value} = \frac{56.1\ (V1 - V2)}{2*M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.9)$$

Where:

V1 = Volume in ml. of N/2 Oxalic Acid used for the blank,

V2 = Volume in ml. of N/2 Oxalic Acid used for the sample

M = Mass in gram of the oil

3.4.2.3. Ester value, content of esters and combined alcohols

The determination of the ester content is of great importance in the evaluation of many essential oils. Most esters, which occur as normal constituents of essential oils, are esters of monobasic acids. Ester value may be defined as "the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1g of the essential oil materials". The value of ester can be calculated as follow:

$$\text{Ester value} = \text{Saponification value} - \text{Acid value} \quad \ldots\ldots\ldots\ldots (3.10)$$

3.4.2.4. Iodine value

0.2g of rosemary essential oil was weighed into a conical flask. 10ml of carbon tetrachloride and 20ml of the wij's solution were added to the flask and the solution was kept in dark for 30min at room temperature. 15ml of 10% potassium iodide solution with 100ml of distilled water were added to the flask. The resulting solution was titrated against 0.1M sodium thiosulphate ($Na_2S_2O_3$), using starch as indicator till the end point where the blue black coloration becomes colorless. A blank titration was carried out at the same time starting with 10ml carbon tetrachloride. Iodine value was then calculated by the following formula.

$$\text{Iodine number} = \frac{[(B-S)*N*12.69]}{\text{Weight of the sample}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.11)$$

Where:

B = 0.1 N Sodium Thiosulfate required (ml) by blank

S =0.1 N Sodium Thiosulfate required (ml) by sample

N = Normality of Sodium Thiosulfate solution

Iodine value can also calculate using empirical formula (Dembirbes, 1998) for determination of higher heating value using the following equation:

$$\text{Iodine value (IV)} = \frac{\text{HHV} - 49.43 + 0.041\ (\text{SN})}{0.015} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.12)$$

Where:

HHV = 0.0317 v + 38.053

v = viscosity of rosemary oil Iodine value

SN = Saponification number

3.4.2.5.Gas Chromatography-Mass Spectroscopy

The component identification was achieved by the GC-MS analysis using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German). Helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl was employed, injector temperature 250℃ and Ion-source temperature 280℃. The oven temperature was programmed from 50℃ (isothermal for 4min.), with an increase of 3℃/min, to 280℃ and held for 10 min. isothermal at 280℃.Total GC running time was 90.67 min.

3.5. Experimental design

3.5.1. Full factorial design

For the analysis of the data, Design Expert Software (version 7.0.0) was used. General Factorial Design had been chosen than other design methods. Because it enables evaluation of the effect of several process parameters and their interactions on the response variable. At each combination of the process setting, the yields of rosemary essential oil were recorded.

This experimental design software was made to:

- ✓ Screen out insignificant factors and identify significant factors.
- ✓ Get some idea about the existence of interaction effects.
- ✓ Understand main effects and get complete information about 2-factor interaction.
- ✓ Characterize how the significant factors affects the response (for optimization purposes)
- ✓ To develop the mathematical model that will describe the effects of the main and interaction factors on the response.

For steam distillation extraction, we had two factors : time and drying conditions. Three levels for drying conditions and four levels for extraction time. Totally 36 experiments were conducted. Temperature and pressure were fixed due to uncontrollable of steam distillation. Even though temperature and pressure are the main factors for steam distillation in addition to time and drying condition, the best or recommended temperature and pressure was selected. That is temperature around the boiling point of water in Addis Ababa and pressure at atmospheric pressure.

4. Results and Discussion

4.1. Introduction

The effects of operating parameters such as drying conditions and extraction time on the quantity and quality of essential oils extracted by means of steam extraction were studied using Rosemary leaves as the plant material. The aspects of the results studies here include both quantitative and qualitative analyses obtained by laboratory analysis and Gas Chromatography Mass spectroscopy. To thoroughly investigate the effect of operating parameters on the yield and quality of oil extracts, the focus area in the analysis of the results was directed towards the following points:

- ✓ Quantity of the oil extracted (yield)
- ✓ Quality of the oil extracted (characterization and composition of the oil)

4.2. Quantitative Analysis of the essential oil

4.2.1. Determination of moisture content of rosemary leaves

Five samples of each kind these are fresh, shade dried and sundried rosemary leaves were taken for this analysis. The samples were taken randomly and average moisture content was calculated using equation (3.1). Table 4.1 indicates fresh leaves moisture content.

Table 4.1 Moisture content of fresh rosemary leaves

	Sample mass (g)				
Time(Hr)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial mass	26.00	27.00	26.50	27.30	43.00
Mass after 2h	14.58	15.65	13.34	14.98	29.12
Mass after 4h	13.34	14.62	12.91	14.93	28.98
Mass after 6h	13.21	14.48	12.83	14.82	28.92
Mass after 8h	12.80	14.41	12.80	14.81	28.07
Mass after 10h	12.80	14.41	12.80	14.81	28.07
Moisturecontent (%)	50.77	46.63	51.70	45.75	34.72

The wet basis moisture content of the five rosemary leaves (*Rosemary Officinalis*) sample were 50.77%, 46.63%, 51.70%, 45.75% ,and 34.72% . Thus, the mean average moisture content of the five rosemary leaves samples was 45.91%. This result is somehow less than a study on fresh rosemary leaves that reported a moisture content of 75-80 % (Kajiwan et al., 2016). This is because of so many factors such as difference in geographical locations, production techniques, and plant collection time.

Table 4.2 Moisture content of shade dried rosemary leaves

	Sample mass (g)			
Time(Hr)	Sample 1	Sample 2	Sample 3	Sample 4
Initial mass	42.1	37.00	24.1	26.30
Mass after 2h	41.61	35.76	22.99	25.04
Mass after 4h	41.02	35.61	22.85	24.87
Mass after 6h	40.86	35.51	22.80	24.80
Mass after 8h	40.62	35.51	22.80	24.80
Mass after 10h	40.62	35.51	22.80	24.80
Moisture content (%)	3.64	4.19	5.70	6.03

Figure 4.2 above shows the wet basis moisture content of the four shade dried rosemary leaves samples having mass of 42.1, 37.00, 24.10; 26.30 grams were 3.64%, 4.19%, 5.70%, and 6.03% respectively. Thus, the mean average moisture content of the four samples was 4.89%, that agreed with literature (Kajiwan et al., 2016).

Table 4.3 Moisture content of sundry rosemary leaves

	Sample mass (g)			
Time(Hr)	Sample 1	Sample 2	Sample 3	Sample 4
Initial mass	43.00	37.00	24.00	26.00
Mass after 2 hr	42.81	36.87	23.65	25.86
Mass after 4 hr	42.78	36.67	23.62	25.79
Mass after 6 hr	41.76	36.59	23.61	25.76
Mass after 8 hr	41.62	36.51	22.86	25.12
Mass after 10 hr	41.61	36.50	22.86	25.11
Moisture content	3.34%	1.37%	4.98%	2.97%

Figure 4.3 shows the moisture content of the four sun dried rosemary leaves samples having mass of 43, 37, 24, and 26, grams were 3.34%, 1.37%, 4.98% and 2.96% respectively. Thus, the average moisture content of the four samples was 3.165%. This result agrees to the moisture

content of the dried rosemary leaves *Rosemary officinalis*, which was 3.5% in dry basis (Kajiwan et al., 2016).

4.2.2. Analysis on factors affecting essential oil yield

The experimental design selected for this study is the general factorial design and the response variable is the Yield of Rosemary Essential oil. Three levels for pre-distillation drying and four levels for extraction time and two factors general factorial design was applied. And then 36 experiments were performed for this research work. The two extraction process variables are pre-distillation drying and extraction time. The other factors like temperature and pressure was maintained at their optimum points obtained from the literature. This is because of uncontrollability of the equipment. Optimization of extraction of essential oil by the classical method involves changing one independent variable while maintaining all others at a fixed level. This procedure is extremely time consuming and expensive for a large number of variables. To overcome this difficulty, the experimental design software can be employed to analyze the extraction parameters of rosemary essential oil.

In order to study the combined effect of the above factors, experiments were performed for different combinations of the physical parameters using statistically designed experiments. The application of statistical experimental design techniques in extraction processes could result an improved product yields, closer confirmation of the output response to nominal and targeted requirements, as well as reduced development time and overall costs.

Design Expert 7.0.0 Software program was used for regression and graphical analysis of the data obtained by incorporating the sequential F-test, lack-of-fit test and other adequacy measures for selecting the best model.

To decide about the adequacy of the model for the extraction of rosemary essential oil the three different tests such as the Sequential model, Sum of Squares, Lack of Fit Tests and Model Summary Statistics were carried out and p-values for all the regressions were associated . Prob > F value for the model is lower than 0.05 which indicates that the model is considered to be statistically significant. This means that at least one of the terms in the regression equation had a significant correlation with the response variable.

Designed experiments were carried out to map quantitative effects of the two parameters these are extraction time and drying conditions. The yields of the extraction is calculated from the relation between the essential oil mass obtained and the raw material mass used in the extraction

(Y = extract/raw material %). The yield curve has constructed from essential oil mass extracted in relation to the amount of extraction time and drying condition. In this work, 1kg of rosemary leaves were used for each experiment. The pressure in the boiler for consecutive tests was set at one atm and temperature 95℃. Higher pressures would have resulted in higher temperatures that would destroy components in the essential oil. The yield (quantities) of the oil extracts from 1kg of rosemary leaves are shown in table 4.4 below.

Table 4.4: Average yield of rosemary essential oil steam distillation

Factors		Yield of essential oil (g)				Average extraction yield (%)
Extraction time (min)	Drying condition	Replicates (g)				
		1	2	3	Average	
30	Fresh	3.2	4.5	3.6	3.78	0.378
	Shade dried	6.2	6.5	7.5	6.73	0.673
	Sun dried	10.8	8.4	8.6	9.27	0.927
60	Fresh	4.6	6.1	5.5	5.4	0.54
	Shade dried	6.9	7.8	8.6	7.77	0.777
	Sun dried	11.4	8.8	10.2	10.13	1.013
120	Fresh	4.1	5.2	5.6	4.97	0.497
	Shade dried	8.6	9.9	7.9	8.8	0.88
	Sun dried	9.3	13.2	11.6	11.37	1.137
150	Fresh	4.1	5.2	5.6	4.97	0.497
	Shade dried	8.6	9.9	7.9	8.8	0.88
	Sun dried	9.3	13.2	11.6	11.37	1.137

The maximum extraction of rosemary essential oil was 1.137% at sun dried rosemary leaves for the extraction time of 120min and the minimum yield of essential oil obtained was 0.378% from fresh rosemary leaves at minimum extraction time that is at 30min. Similar result was obtained in literature which made different pre-distillation drying and found 1.0% for sun dried *Rosemary Officinalis* leaves (Ram et.al., march 2011).

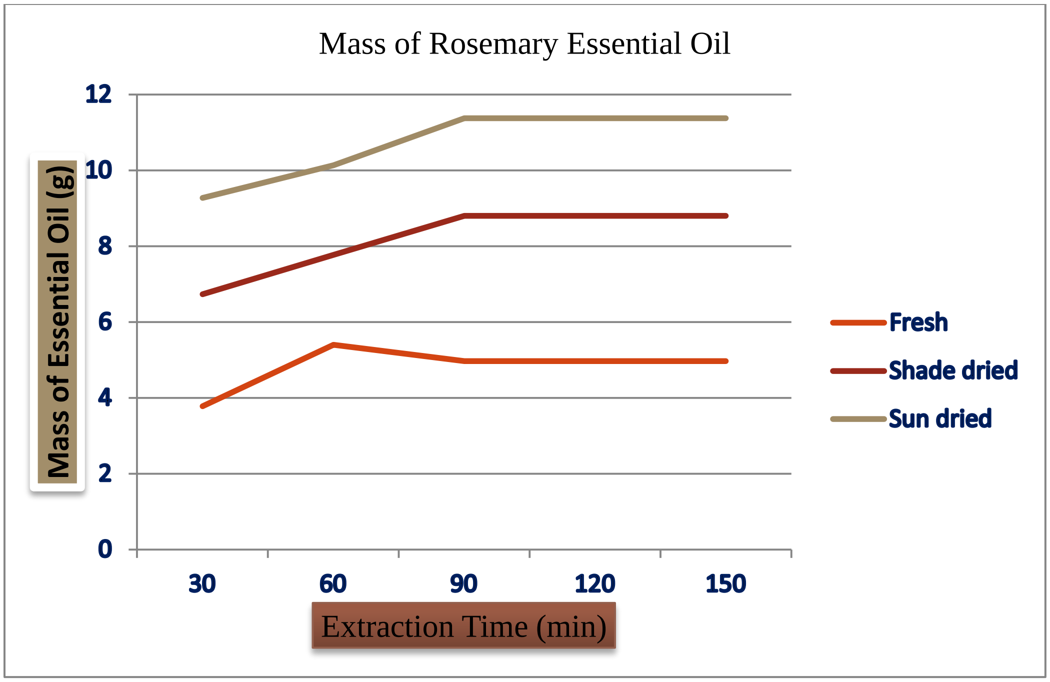


Figure 4.1 Average mass of rosemary essential oil extracted

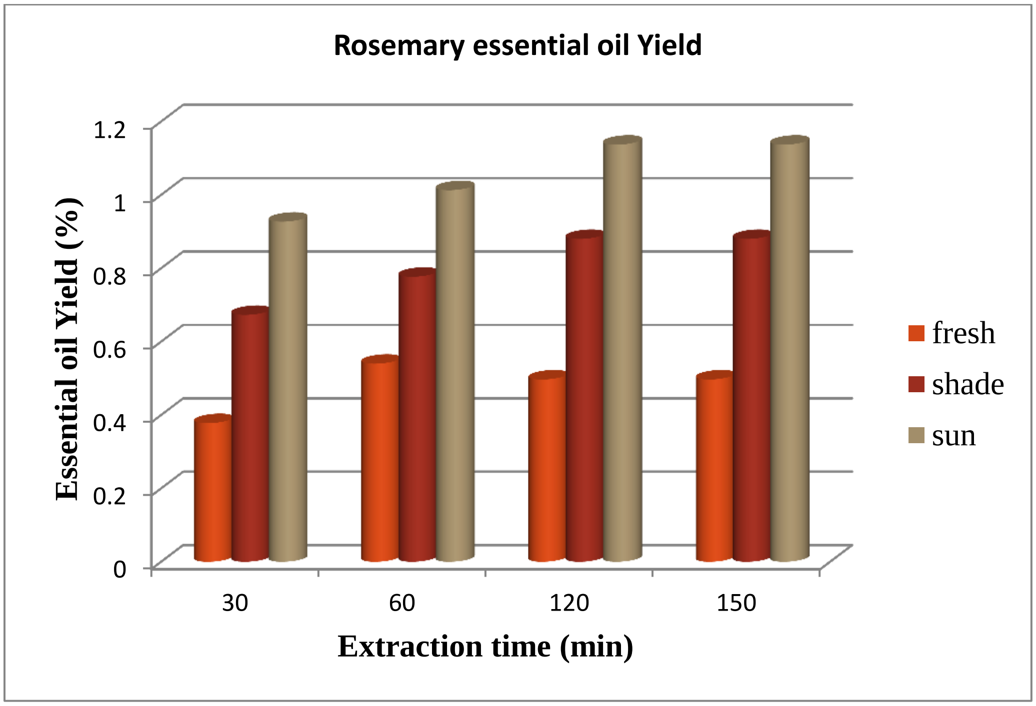


Figure 4.2 Average essentials oil yield in percentage

Figure 4.1 and 4.2 above show the results for the cumulative mass and percentage of rosemary essential oil extract plotted against the extraction time. From this graph, it can be observed that the oil extracted from sun dried rosemary leaves provide the highest overall cumulative mass after 120 minutes of extraction period followed by the oil extracted from shade dried rosemary leaves. On the other hand fresh rosemary leaves gives the list cumulative mass of essential oil. The reason for that (Peisíno et al., 2005) explained as essential oil extracted from the wet plants present fewer components than dry sample due to water molecules solvate the components This result is somehow similar with literatures (Chauhan, March 2011) . It is also evident that there is no more oil coming off at all drying conditions after 120 minutes mark as all cumulative curves appears to flatten off beyond this point. From this result, it can be noticed that maximum extraction time for all drying conditions is about 120 minutes, which ensures the recovery of all the extractable oil components. The quantitative study of the oil used to give an insight on the effect of pre-extraction drying and extraction time on oil extraction during steam distillation process. The quantitative results are bound to be helpful in studying the effect on mass of oil extracted. Table 4.5 shows predicted, actual and residual yield of rosemary essential oil.

Table 4.5 Experimental and predicted percent yield of rosemary essential oil

Run	Pre-extraction drying	Extraction Time (min)	Experimental Yield of Essential Oil (%)	Predicted Yield of Essential oil (%)	Residuals
1	Fresh	30	0.32	0.38	-0.057
2	Fresh	30	0.45	0.38	0.073
3	Fresh	30	0.36	0.38	-0.017
4	Shade dry	30	0.62	0.67	-0.053
5	Shade dry	30	0.65	0.67	-0.023
6	Shade dry	30	0.75	0.67	0.077
7	Sun dry	30	1.08	0.93	0.15
8	Sun dry	30	0.84	0.93	-0.087
9	Sun dry	30	0.86	0.93	-0.067
10	Fresh	60	0.46	0.54	-0.080
11	Fresh	60	0.61	0.54	0.070
12	Fresh	60	0.55	0.54	0.010
13	Shade dried	60	0.69	0.78	-0.087
14	Shade dried	60	0.78	0.78	3.333E-003
15	Shade dried	60	0.86	0.78	0.083

Table 4.5: Cont'd

16	Sun dried	60	1.14	1.01	0.13
17	Sun dried	60	0.88	1.01	-0.13
18	Sun dried	60	1.02	1.01	6.667E-003
19	Fresh	120	0.41	0.5	-0.087
20	Fresh	120	0.52	0.5	0.023
21	Fresh	120	0.56	0.5	0.063
22	Shade dried	120	0.86	0.88	-0.020
23	Shade dried	120	0.99	0.88	0.11
24	Shade dried	120	0.79	0.88	-0.09
25	Sun dried	120	0.93	1.14	-0.21
26	Sun dried	120	1.32	1.14	0.18
27	Sun dried	120	1.16	1.14	0.025
28	Fresh	150	0.41	0.5	-0.087
29	Fresh	150	0.52	0.5	0.023
30	Fresh	150	0.56	0.5	0.063
31	Shade dried	150	0.86	0.88	-0.020
32	Shade dried	150	0.99	0.88	0.11
33	Shade dried	150	0.79	0.88	-0.09
34	Sun dried	150	0.93	1.14	-0.21
35	Sun dried	150	1.32	1.14	0.18
36	Sun dried	150	1.16	1.14	0.025

4.2.3. Development of regression model equation

The model equation that correlates the response (yield of essential oil) to the extraction process variables in terms of actual value after excluding the insignificant terms was given below. The predicted model for percentage of yield of rosemary essential Oil in terms of the coded factors is given in equation (4.1).

Yield of essential oil = +0.78 - 0.30*A[1] + 0.025*A[2] -0.12*B[1] - 1.111E-003*B[2] +0.060 *B[3]……………………… ………..…………………………………..…..(4.1)

Where:

A is pre- distllation drying

B is extraction time

The result indicates that the yield of rosemary essential oil was dependent only on the linear terms. Based on the coefficients in equations (4.1) it was evident that the percentage of essential oil yield increases with the pre-distillation drying (A) and extraction time (B). Extraction time has a more profound effect on rosemary essential oil yield as compared to pre-distillation drying. As expressed in eq. (4.1), the pre-distillation drying and extraction time show significantly linear effects on the essential oil yield, which was clearly indicated by the largest positive linear regression coefficient 0.060 and 0.025 respectively. While fresh rosemary leaves A [1] content possessed the negative effect with the linear coefficient of
(-0.30).

4.2.4. Model adequacy check

The model was tested for adequacy by analysis of variance. The regression model was found to be reasonably significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared having a value of 0.8884, 0.8372 and 0.7488 respectively. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.8884. It implies that 88.84% of the total variation in the yield of essential oil was attributed to the experimental variables studied. The “Pred R-Squared” of rosemary essential oil 0.7488 is close to the "Adj R-Squared" of 0.8372 that is less than 0.2 differences as one might expect. The “Adeq Precision" measures the signal to disturbance ratio due to random error. A ratio greater than four is desirable. Rosemary essential oil has ratio of 12.382, which indicates an adequate signal. Therefore, this model can be used to navigate the design space. The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure (4.3).

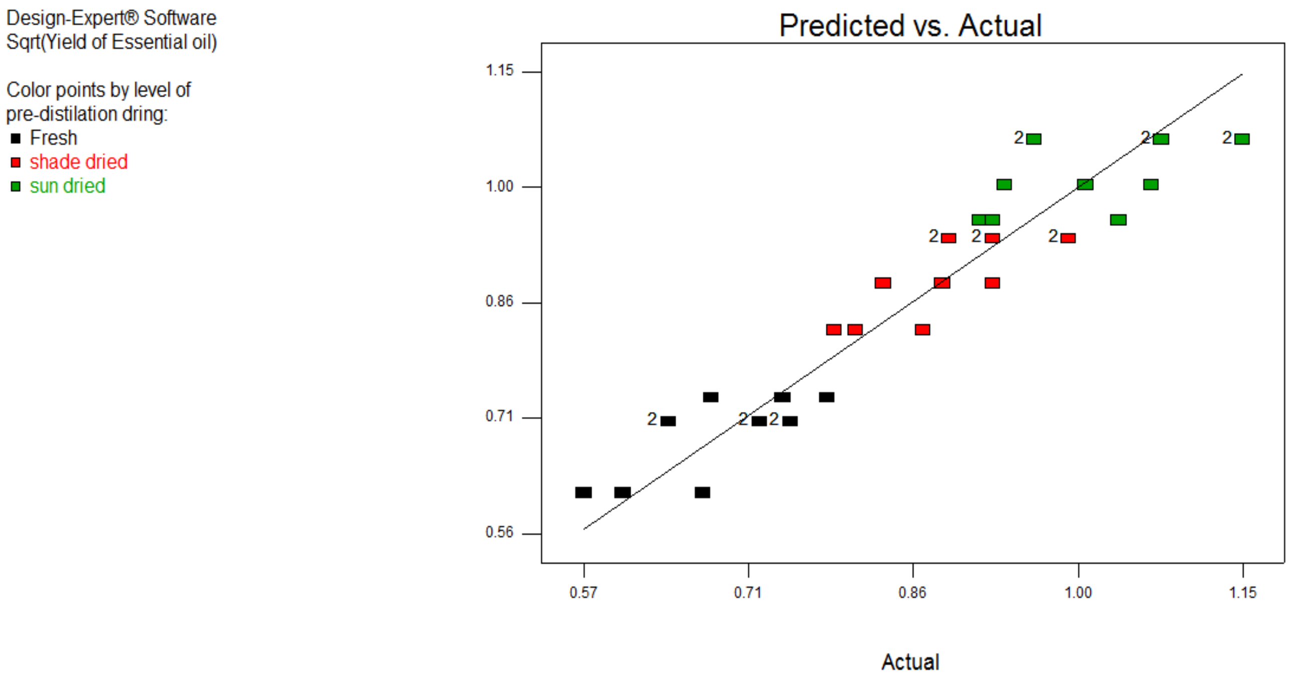


Figure 4.3 Predicted versus actual values of rosemary essential oil.

The results in Figure (4.3) above demonstrates that the regression model equation provided accurate description of the experimental data, in which all the points are close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the three-replication process variables to the yield of essential oil.

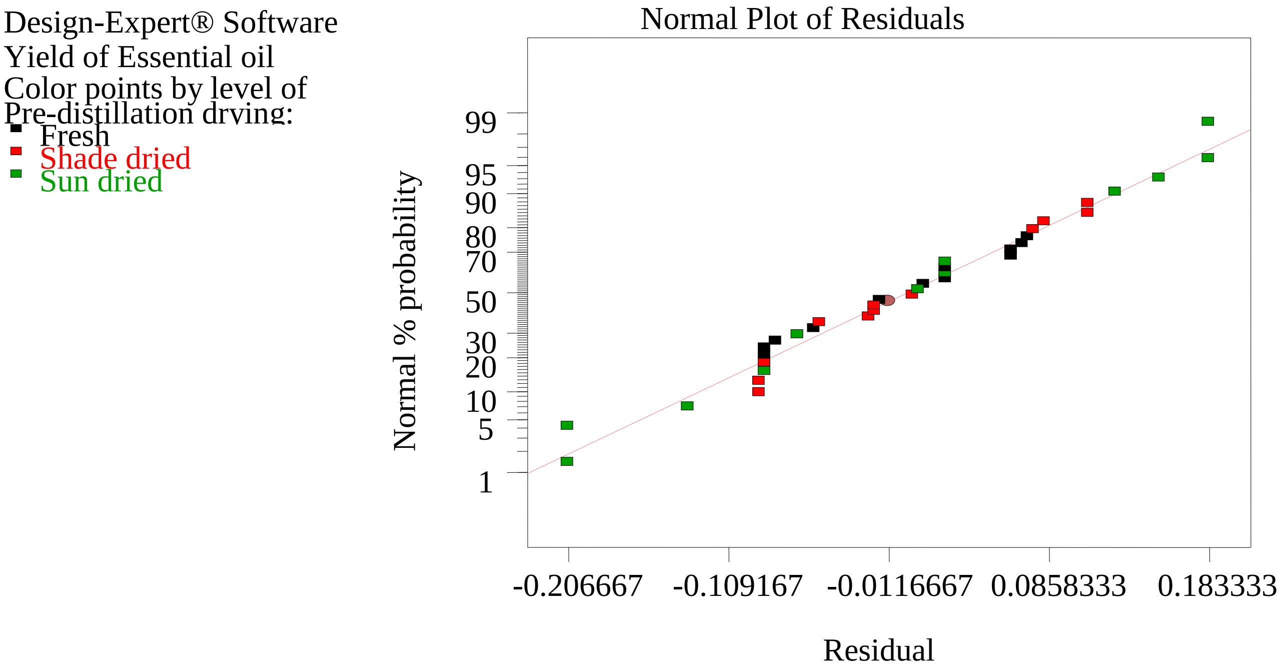


Figure 4.4 Normal plots of residuals of rosemary essential oil

Figure 4.4 shows how precisely the rosemary essential oil yield is modeled, because all the points line up well and the deviation of points for essential oil yield from normality is insignificant. In addition, the normal probability plot indicates the residuals following a normal distribution, in the case of this experiment the points in the plots shows fit to a straight line in the figure i.e. the error distribution is approximately normal.

The adequacy of the model was further checked by analysis of variance (ANOVA) as shown in Table (4.6). Based on a 95% confidence level, F-value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is likely that any of the factors have a significant effect on the response with the P-value less than 0.05. It was calculated by model mean square divided by residual mean square. Here the model F-value of 17.36 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to personal error or disturbance.

Table 4.6 ANOVA for the regression model equation and coefficients

Source	Sum of Square	Df	Mean Square	F-value	P-value Prob > F	Remark
Model	0.76	11	0.069	17.36	<0.0001	significant
A-Pre-extraction drying	0.69	2	0.34	86.04	<0.0001	significant
B-Extraction time	0.065	3	0.022	5.40	0.0055	significant
AB	0.011	6	1.785E-003	0.45	0.8392	
Pure Error	0.096	24	3.985E-003			
Core Total	0.86	35				
Lack of Fit	0.010	2	5.163E-003	0.37	0.6927	Not significant

Table 4.6 shows values of "Prob > F" less than 0.050 indicate model terms are significant. In this case A, B, are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This shows that the pre-extraction drying and extraction time are significantly affect the essential oil yield but the interaction effect is not as such effect on rosemary essential oil yield. The "Lack of Fit F-value" of 0.37 implies the Lack of Fit is not significant relative to the pure error. There is a 69.27% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit.

4.2.5. Effect of Individual Process Variables

Based on the analysis of variance, rosemary essential oil yield was significantly affected by various process variables. Significant individual process variables that affect the yield are pre-distillation drying and extraction time. These factors have influence on the yield of rosemary essential oil.

4.2.5.1. Effect of pre-distillation drying on the percent yield of essential oil:

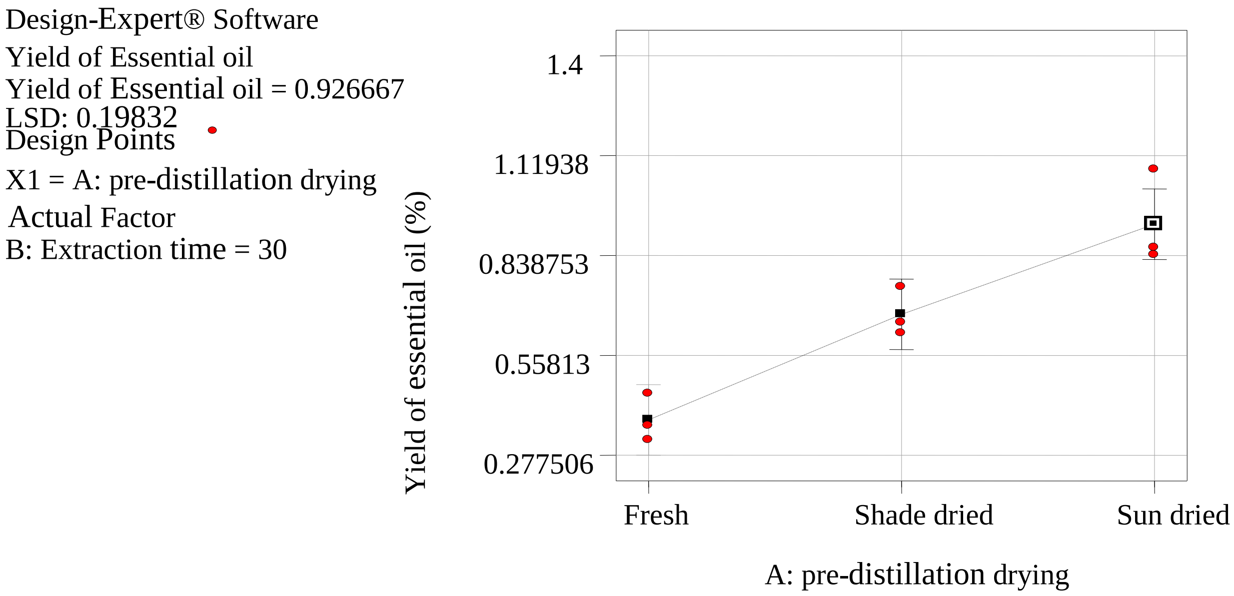


Figure 4.5 Effect of pre-distillation drying on yield of essential oil at 30min

Figure 4.5 above shows maintaining the extraction time constant at 30min and changing the pre-distillation drying, the yield of rosemary essential oil increases as the rosemary leaves getting dried. From this graph maximum average yield of rosemary essential oil was obtained from sun dried rosemary leaves, which is 0.926%. Whereas the minimum average yield of essential oil was fresh rosemary leaves.

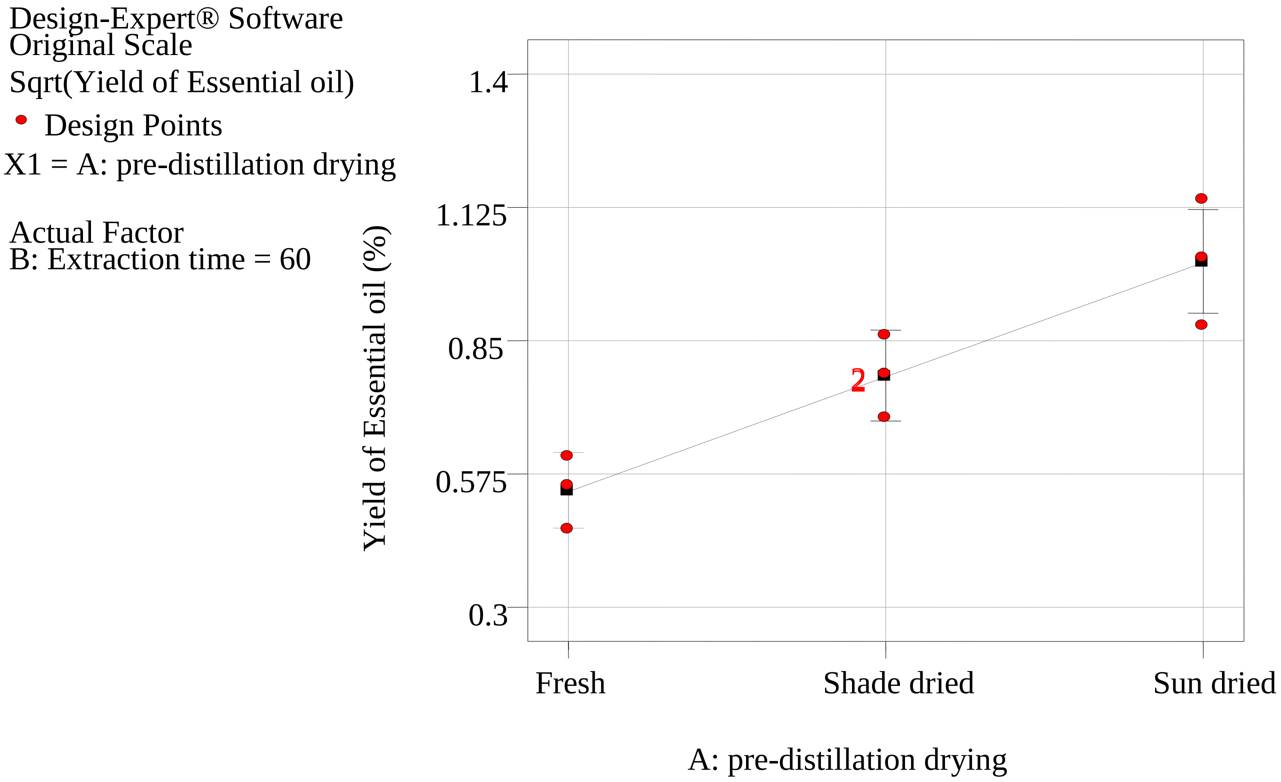


Figure 4.6 Effect of pre-distillation drying on yield of essential oil at 60 min

Figure 4.6 above shows maintaining the extraction time constant at 60 min and changing the pre-distillation drying, the yield of rosemary essential oil increased as the rosemary leaves pre-distillation drying conditions move from fresh to sun dried rosemary leaves. At this point maximum average yield of rosemary essential oil was obtained from sun dried rosemary leaves, which is 1.010%. Whereas the minimum average yield of essential oil was obtained from fresh rosemary leaves.

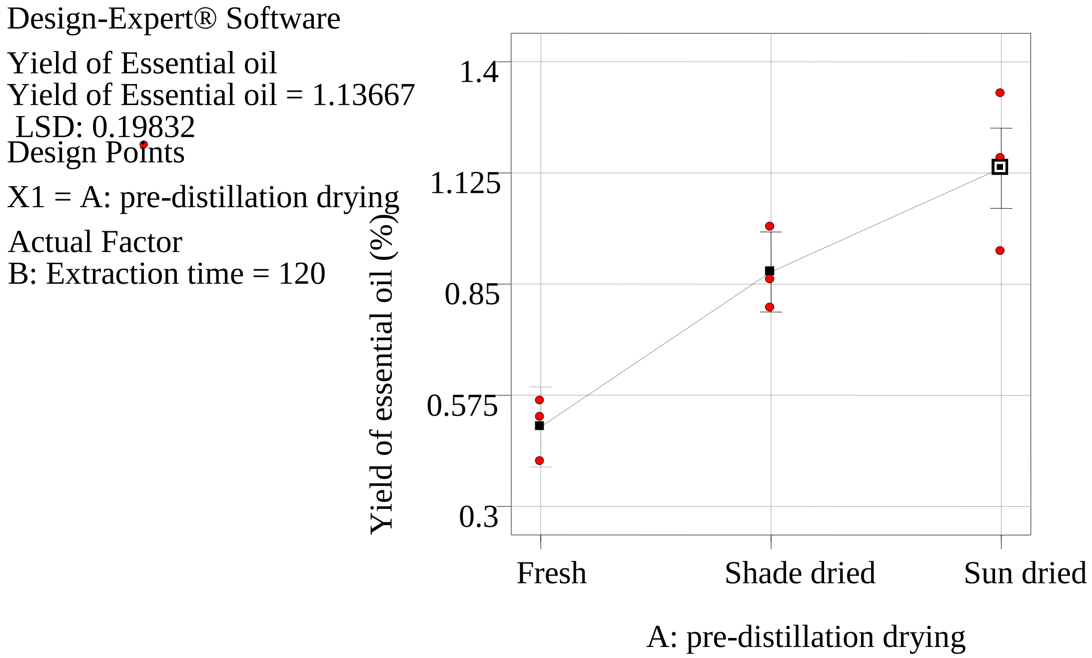


Figure 4.7 Effect of pre-extraction drying on yield of essential oil at 120 min

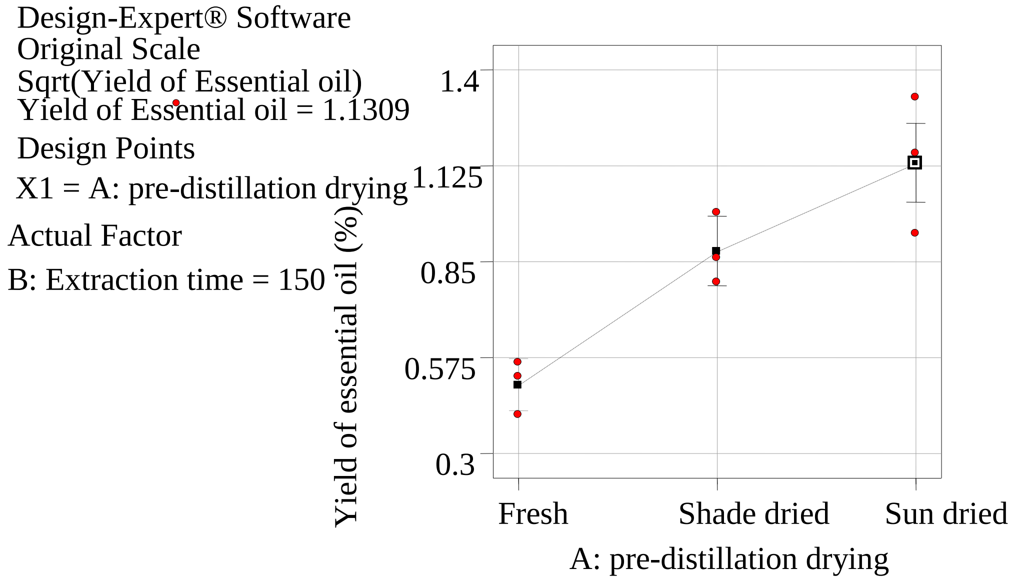


Figure 4.8: Effect of pre-extraction drying on yield of essential oil at 150 min

Figure 4.7 and figure 4.8 are exactly the same graph as shown above. Here the extraction time was kept at 120 and 150 min and in both cases the yield of rosemary essential oil was increased as pre-distillation drying conditions increased from fresh leaves to sun dried rosemary leaves. At this time, the maximum yield of rosemary essential oil was 1.131% from sun dried rosemary leaves. Those two figures also indicate that further extraction of rosemary essential oil above 120 min is meaningless. Therefore, the maximum extraction time of rosemary essential oil from steam distillation in AAiT laboratory is 120min.

4.2.5.2. Effects of extraction time on yield of essential oil

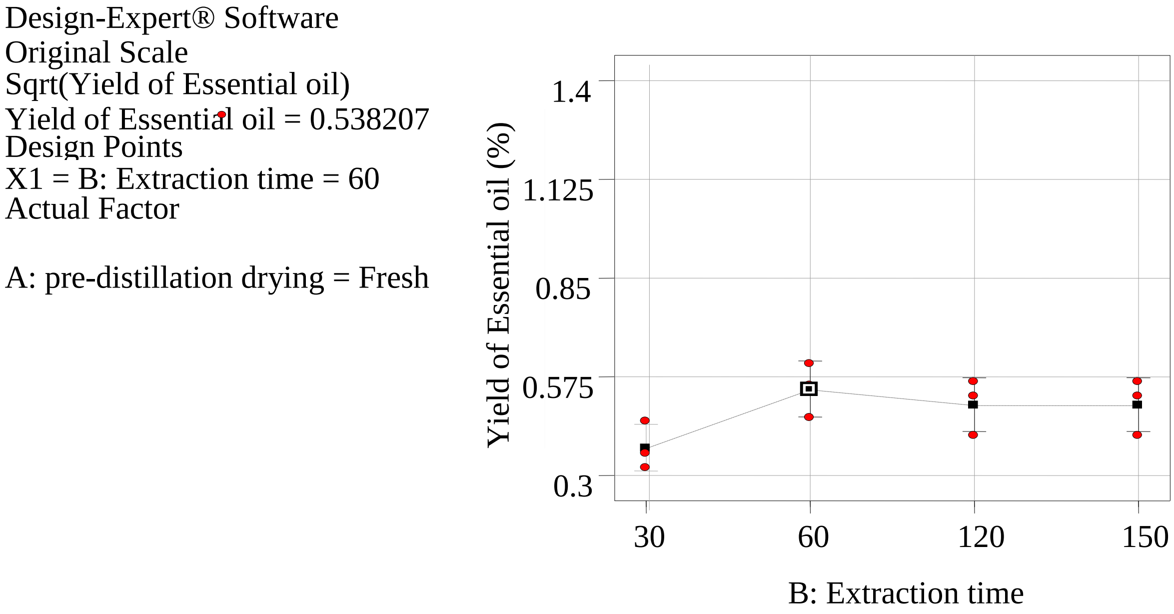


Figure 4.9 Effects of extraction time on yield of essential oil for Fresh leaves

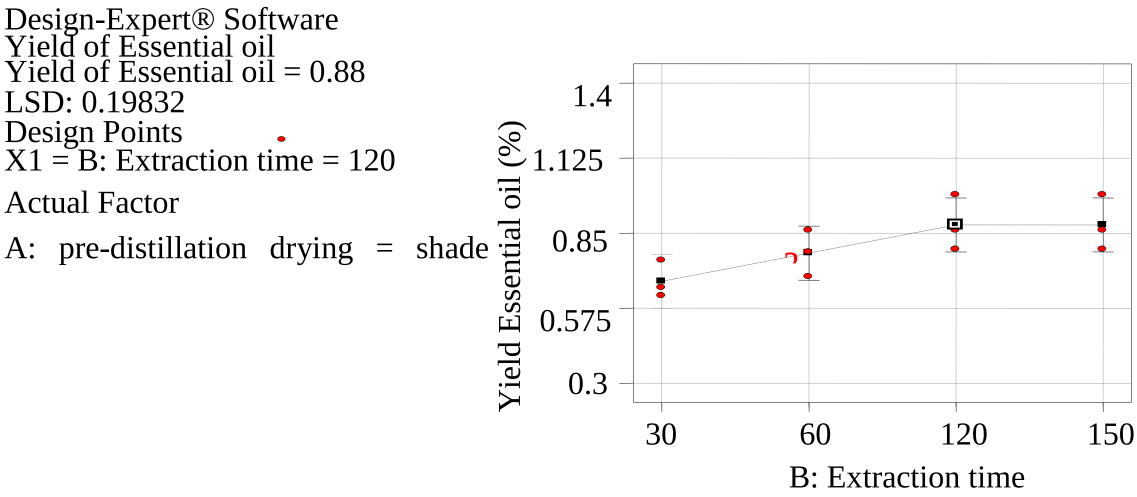


Figure 4.10: Effects of extraction time on yield of essential oil for shade dried leaves

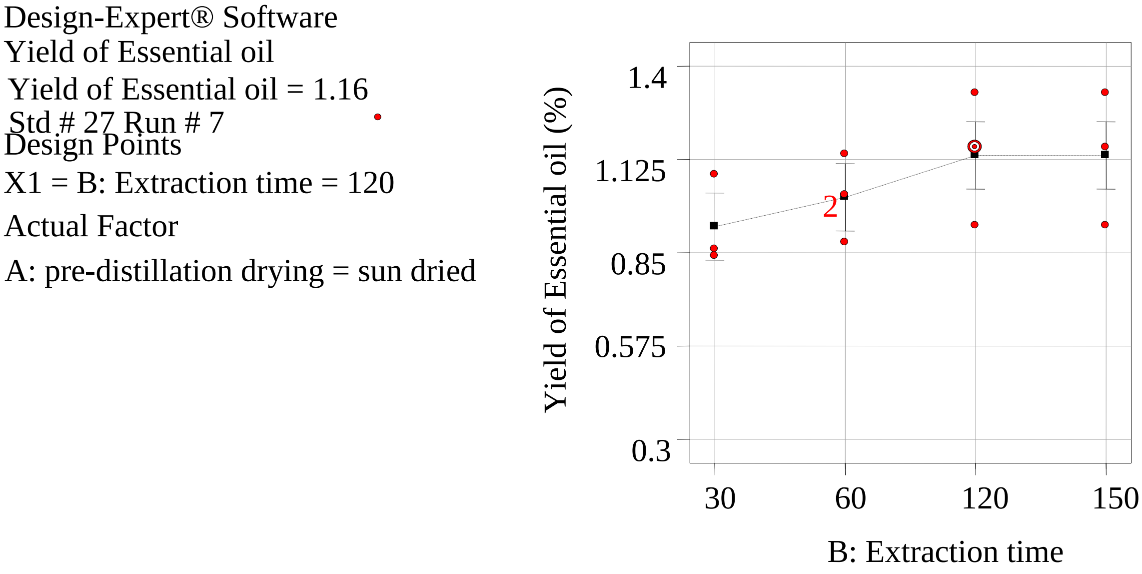


Figure 4.11: Effects of extraction time on yield of essential oil for sun dried leaves

Figure 4.9, 4.10 and 4.11 above indicates the effect of extraction time at different pre-distillation drying conditions. From these figures it is clearly shows that increasing the extraction time will increase the yield of rosemary essential oil. This is because increasing the time will increase the temperature of the oil glands where essential oils located, so more essential oils will move to the surface of the oil gland and evaporated by the coming steam from the boiler. For all the three drying conditions, the maximum extraction time from AAiT steam distillation was 120 min. here also see from the three figures 83-90% of the rosemary essential oil was extracted below 60 min.

4.2.5.3. Effects of interactive parameters

From design, expert soft ware the output of interaction effect between pre-distillation drying and extraction time is shown below figure 4.12.

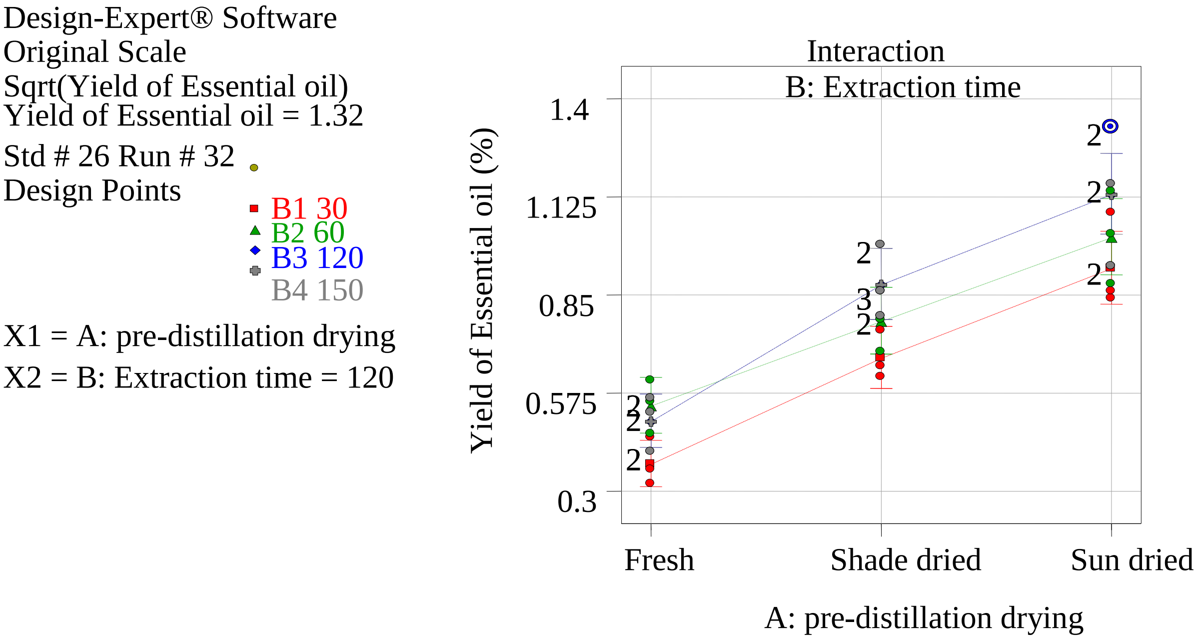


Figure 4.12 Interaction effects of the pre-distillation drying and extraction time

Where: B1, B2 and B3 and B4 are codes for extraction time for 30, 60,120, and 150 min with four different colors red, green, blue, and ash respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response. Design-expert was applied to analyze results on the extraction process and a first order regression equation, with the interaction terms of the form.

4.3. Physical and chemical characteristics of rosemary essential oil

4.3.1. Physical characteristics of rosemary essential oil

4.3.1.1. Specific gravity

Density bottle method was used to determine the specific gravity of the essential oil as detail experimental procedure were stated in chapter three .From eq.(3.5)

W_0 is weight in gram of empty Pycnometer at 20℃ = 18.73g

W_1 is weight in gram of Pycnometer with sun dried rosemary oil at 20℃ = 41.11g

W_2 is weight gram of water in Pycnometer @ 20℃ = 43.97g

Substituting the above values into eq. (3.5) gives:

$$\text{Sp.gr} = \frac{W_1 - w_0}{W_2 - w_0} = \frac{41.11 - 18.73}{43.97 - 18.73} = 0.8867$$

Density of sun dried rosemary essential oil =Specific gravity × density of water

$= 0.8867 \times 1 g/ml$

= 0.8867 g/ml

Density of shade dried rosemary essential oil = 0.7547 g/ml

Density of fresh rosemary essential oil = 0.6847 g/ml

4.3.1.2. Kinematic viscosity

Dynamic viscosity of essential oil, which was read from vibro-viscometer, was 0.96 mPa.s, 0.95 mPa.s and 0.92mPa.s for Sun dried, Shade dried and Fresh rosemary essential oil respectively at temperature 19.5℃. Substituting the Sun dried dynamic viscosity of the oil which is 0.96mPa.s = 0.96×10^{-3} *kg/m.s* and density = 886.7 *kg/*m^3 in to equation (3.6) gives the kinematic viscosity.

$$\text{Kinematic viscosity} = \frac{0.96 * \frac{10^{-3} kg}{m.s}}{886.7 kg/m^3} = 1.0827 * 10^{-6} m^2/s$$

Kinematic viscosity of shade dried rosemary essential oil = $1.2588 * 10^{-6} m^2/s$

Kinematic viscosity of fresh rosemary essential oil = $1.3436 * 10^{-6} m^2/s$

4.3.1.3. pH value

The pH value of rosemary essential oil determined by pH electrode as measuring experimental procedure was stated in chapter three.

Table 4.7 PH value of Rosemary essential oil

Types of essential oils	pH values				
	1	2	3	4	Average
Sun dried	4.01	3.93	4.26	4.41	4.15
Shade dried	4.98	5.73	6.25	5.96	5.75
Fresh	6.01	5.89	6.42	6.33	6.16

The average potential of Hydrogen (pH) values of rosemary essential oils are 4.15 for sun dried, 5.75 for shade dried and 6.14 for fresh leaves.

4.3.1.4. Optical rotation

Optical rotation was determined in College of natural science in Addis Ababa University physics department. The average optical rotation for the three-pre drying conditions are +8,+9 and +11 for Fresh ,shade dried and Sun dried rosemary essential oil respectively. These results are in the range that is found from the standard characteristics of rosemary essential oil. It says the Optical rotation of rosemary essential oil is best between -3° and +18°. There are also international journals who found the optical rotation of *Rosmarinus officinalis* between 11.75° to 12.13°. (Ana Cristina et,al., 2005). Figure 4.13 shows the photograph of spectrometer.

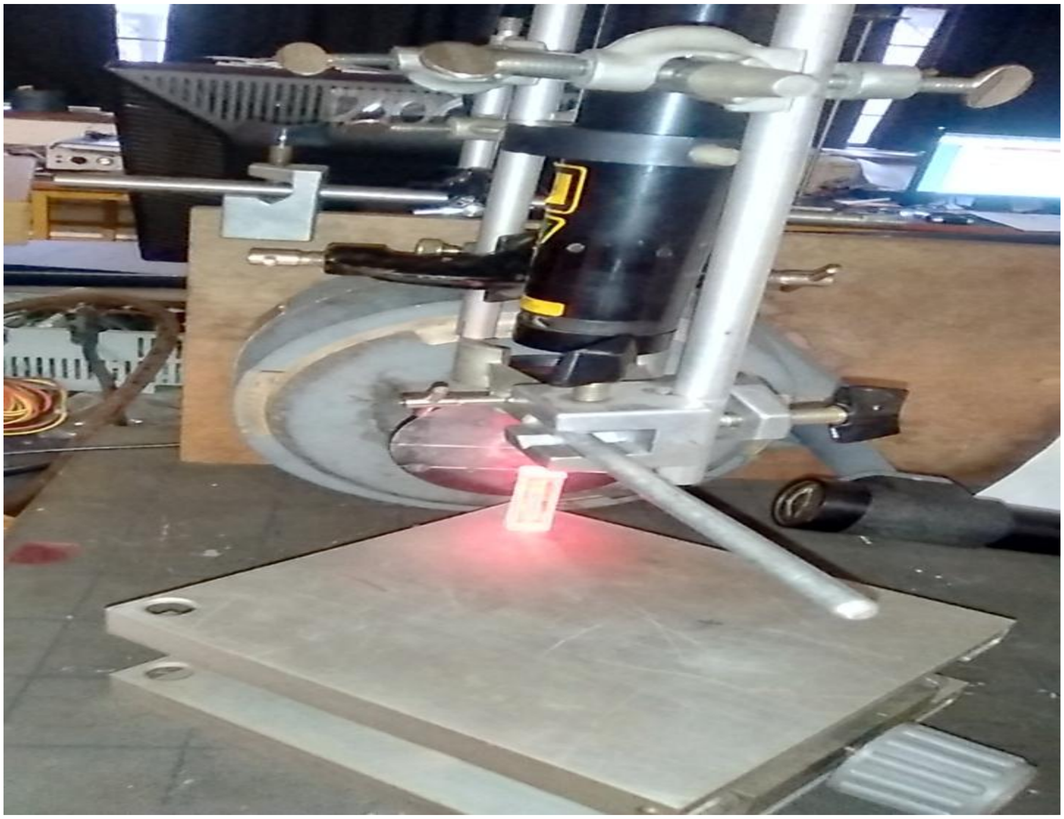


Figure 4.13 Spectrometer, optical rotation measuring instrument

4.3.1.5. Refractive index

The value refractive index of rosemary essential oil was determined by spectrometer using Brewster's angle. Brewster's angle (θ_b), is the angle at which the glare off the gels surface is the most polarized, thus allowing for a maximum amount of light to be removed by the polarizer.

It was found that sun dried, Shade dried and Fresh rosemary leaves essential oil have the Brewster's angle equal to 55.76°, 55.74°,55.75° respectively. Put these values in to equation (3.7) and calculate refractive index, N.

$$N = tan\theta_b = \tan 55.76 = 1.4697$$

$$N = tan\theta_b = \tan 55.74 = 1.4682$$

$$N = tan\theta_b = \tan 55.75 = 1.4689$$

There for the refractive index of the sun dried, Shade dried and Fresh rosemary essential oil are 1.4697, 1.4682, and 1.4689 respectively at 25℃.which is almost closed to the study that reported the refractive index of rosemary essential oil is 1.4689 (Ana Cristina et,al., 2005). Table 4.8 summarized the main physical characteristics of rosemary essential oil.

Table 4.8 Summary of physical characteristics of extracted rosemary essential oil.

Physical properties	Values for fresh rosemary essential oil	Values for Shade dried Rosemary essential oils	Values for sun dried rosemary essential oils	Unites
Color	Light yellow	Light yellow	Light yellow	-
Odor	Camphoraceous	Warm camphoraceous	Warm camphoraceous	
Boiling point at room temperature	156.5	162.3	160.9	℃
Solubility	In soluble in water	In soluble in water	In soluble in water	
Specific Gravity	0.8867	0.7547	0.6847	g/cm^3
Flash point	56	48	47	℃
Vapor residuals after 100℃	92	95	94	%
Dynamic viscosity	0.92	0.95	0.96	mPa.s
pH value	6.16	5.75	4.15	-
Optical rotation	8	9	11	°
Refractive index @ 25℃	1.4689	1.4682	1.4697	-

4.3.2. Chemical characteristics of rosemary essential oil

4.3.2.1. Acid value

Table 4.9 Acid value of rosemary essential oil at different raw material conditions.

Raw material conditions	Weight of sample(g)	Volume of Sodium Hydroxide used(ml)	Acid value
Freshly extracted Oil	2	2.12	5.94
Shade dried extracted Oil	2	0.99	2.77
Sun dried extracted Oil	2	0.89	2.49

Table 4.9 shows the average Acid value of the three pre-distillation drying conditions which are fresh , shade dried and sun dried *Rosmarinus officinalis* are 5.94, 2.77, and 2.49 respectively. All the acid values are in the range compared with other studies (Mohamed, 2014).

4.3.2.2. Saponification value

From equation (3.9) Saponification value of the rosemary essential oil was calculated and the values are stated in table 4.10 below.

Table 4.10 Saponification values of rosemary essential oil

Raw material Condition	Volume of Oxalic acid for the blank(ml)	Volume of Oxalic acid for sample(ml)	Mass of the sample(g)	Saponification value
Freshly extracted oil	20.3	18.6	2	23.8
Shade dried extracted oil	16.9	15.6	2	18.2
Sundried extracted oil	15.3	14.6	2	9.8

The average Saponification value of the three pre-distillation drying conditions: Fresh, Shade dried and Sun dried *Rosmarinus officinalis* are 23.8, 18.2, and 9.8 respectively. Different literatures obtained different Saponification values.

4.3.2.3. Percentage of free fatty acid (% FA)

Free fatty acid was determined using the following equation:

$$\%\text{FA} = \text{K x Acid Value (AV)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(4.1)}$$

Where: K = Constant (0.503) (Soni, Dabhi, & Thomas, n.d.)

AV= is acid value of the oil

$$\%\ \text{FA} = 0.503 \times \text{AV} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{..(4.2)}$$

Table 4.11 Free fatty acid values of rosemary essential oil

Raw material condition	Acid Value (AV)	% FA
Freshly extracted oil	5.94	2.9878
Shade dry extracted oil	2.77	1.3933
Sundry extracted oil	2.49	1.2524

4.3.2.4. Iodine value

The iodine value (IV) is the amount of iodine (in grams) necessary to saturate 100g of oil sample. The iodine value is used to determine the unsaturated of oils and in assessing the stability of oil in industrial application .The lower the iodine value of oil, which reflects its characteristics such as higher resistance to oxidation, the longer shelf life and higher quality. Whereas the higher the iodine value of oil, the lower the quality. Iodine values of rosemary essential oil at different raw material condition were calculated using equation (3.11).

$$\text{Iodine number} = \frac{[(\text{B}-\text{S})*\text{N}*12.69]}{\text{Weight of the sample}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.2)$$

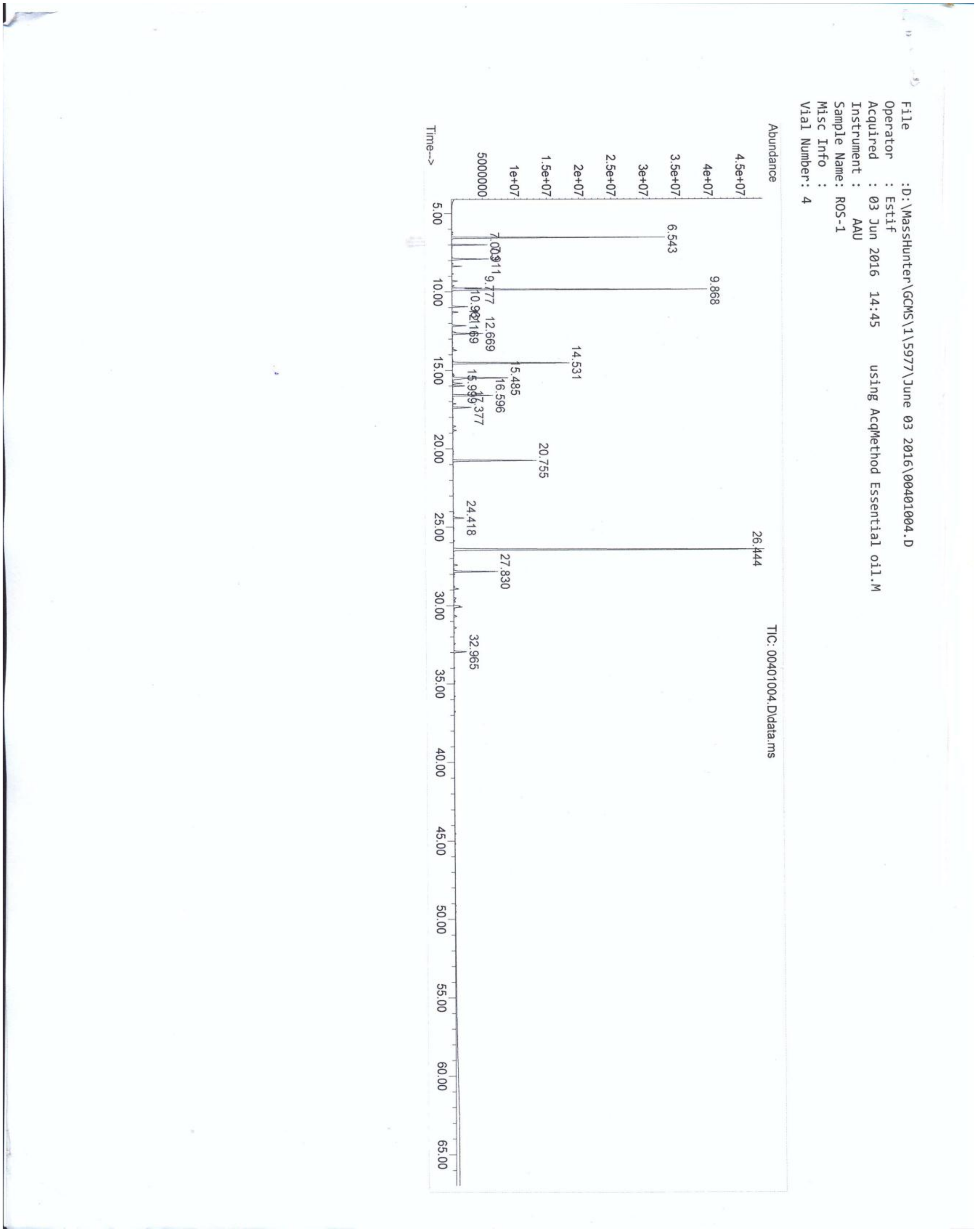
Table 4.12 Iodine value of rosemary essential oils

Raw material condition	Iodine Value
Freshly extracted oil	109.6
Shade dried extracted oil	123.7
Sun dried extracted oil	119.4

Decrease in iodine value indicates lipid oxidation and this might be due to metallic ions present among other factors, which enhances or promotes oxidation. The above Iodine Value numbers are almost closed to the values obtained from journals (Barkatullah et al., 2012). which obtained the iodine value of 102 to 118 for different essential oils.

4.3.2.5. Gas Chromatography- Mass Spectroscopy

A total of eighteen components, with different retention times, were eluted from the GC column as indicated by the chromatogram (figure 4.14) and were further analyzed with an electron impact Mass Spectroscopy voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in table 4.13. The relative amount of individual components was calculated based on GC peak areas. Comparison of the GC-MS spectrograph obtained with the instruments data bank together with NIST MS data demo version revealed that the essential oil of *Rosemarinius officinalisis* contained a mixture of terpenes that eluted at different retention times depending on the boiling point of the eluted component.



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File        :D:\MassHunter\GCMS\1\5977\June 03 2016\00401004.D
Operator    : Estif
Acquired    : 03 Jun 2016  14:45     using AcqMethod Essential oil.M
Instrument  :    AAU
Sample Name: ROS-1
Misc Info   :
Vial Number: 4
```

Figure 4.14 Chromatogram of *Rosmarinus officinalis*

Table 4.13 Chemical composition of rosemary essential oil steam distillation

Pick No.	Components	Library Match Quality (%)	Retention Time(min)	Area (%)
1	2,6,6-Trimethylbicyclo [3.1.1] hept-2 ene	97	6.543	12.08
2	Bicyclo [3.1.1] heptane,6,6-dimethyl 1-2-methylene	97	7.003	2.14
2	Beta pinene	94	7.003	2.14
4	D-Limonene	99	9.777	1.98
5	Eucalyptol	99	9.868	17.06
6	Gamma – Terpinene	96	10.961	1.09
7	Cyclohexene,1-methyl-4-(1-methyle thylidene)	98	12.169	1.00
8	Linalool	93	12.669	2.13
9	Camphor	98	14.531	9.14
10	Endo-Borneol	97	15.485	5.07
11	Terpinen-4-ol	95	15.999	0.96
12	Terpineol	91	16.596	3.08
13	Bicycle[3.1.1]hept-3-en-2-one,4,6,6-trimethyl	98	17.377	1.53
14	Bornyl acetate	99	20.755	6.79
15	Alpha.-ylangene	98	24.418	0.97
16	Caryophyllene	99	26.444	27.47
17	Humulene	97	27.830	3.85
18	Caryophyllene oxide	90	32.965	1.22

Result obtained by GC-MS analysis of the essential oils of *Rosmarinus officinalis* L. are presented .The identification of the components of the essential oil extracted by steam distillation process was carried out by comparison of their mass spectra and retention times to those of reference standards. From this table 4.13, it can be seen that these oils were characterized by the presence of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes, but the quantitative differences were observed in the contents of these components. The steam distillation oil composition is comparable to those reported in literatures. This oil has high monoterpene hydrocarbons and other contents. Thirty-two components were identified in the essential oil of *Rosmarinus officinalis.* Monoterpene hydrocarbons were found to be the major group Of compounds these are α-pinene (12.08 %) followed by camphor (9.14%). The major component was α-pinene (12.08 %). Other predominant components were camphor (9.14 %), camphene (2.14 %), β-pinene (2.45 %), bornyl acetate (6.79 %), limonene (1.98 %), borneol (5.07%), and α-terpineol (3.08%) .The chemical compositions revealed that these leaves had composition somehow closed to those of other *Rosmarinus officinalis* essential oils GC-MS analyzed data. For example International Journal of Pharmacy and Pharmaceutical Sciences reported the following values α -pinene (18.25%), camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%) (Elhoussine et al., 2011).

5. Conclusions and Recommendations

5.1. Conclusions

This work was intended to study the influence of different factors (pre-distillation drying condition and extraction time) on the quality and quantity of extraction of rosemary essential oils. Temperature, pressure and particle size was maintained constant at their optimum conditions from the literature due to the uncontrollability of the equipment. Temperature was set at 95℃ and pressure was at one atmosphere. Variability of these operating conditions is the pre-dominant factors for the quality and quantity of the essential oil. There are different methods of essential oil extraction from rosemary leaves. However, in this study steam distillation extraction method was used due to availability of the equipment and it was recommended by so many literatures. The percentage of essential oil yield in sun dried rosemary leaves samples is the highest as compared to shade dried and fresh rosemary leaves samples. The reason for that (Peisíno et al., 2005) explained as essential oil extracted from the wet plants present fewer components than dry sample due to water molecules solvate the components. The maximum oil yield obtained was 1.13% for sun dried rosemary leaves at 120 min of extraction time followed by shade dried rosemary leaves. It was 0.88% at the same extraction time. The minimum yield was 0.37% from fresh rosemary leaves at 30min of extraction time. It was clearly seen in design expert software that decreasing moisture content of rosemary leaves would increase the yield at any extraction time. So many physical and chemical properties like specific gravity at 25℃, dynamic viscosity, kinematic viscosity, pH value, flash point, refractive index, optical rotation ,solubility in ethanol, acid value, Saponification value, ester value, iodine value and aldehyde content of the rosemary essential oil was determined and obtained comparable results from literatures. Furthermore, the components of rosemary essential oil were determined using Gas Chromatography -Mass Spectroscopy (GC-MS), and eight major components were identified. Monoterpene hydrocarbons were found to be predominant. The major components were α-pinene (12.08 %), camphor (9.14 %), camphene (2.14 %), bornyl acetate (6.79 %), borneol (5.07%), and α-terpineol (3.08%). These components are very important for cosmetics and antioxidant activities.

5.2. Recommendations

Based on this research findings, the following recommendations are forwarded.

- In this research the effect of temperature and pressure factors were not studied. This is due to uncontrollability of equipment of steam distillation that used for extraction of essential oil. Therefore, further study should be done in order to produce much butter quantity and quality of rosemary essential oil.
- More pre-distillation drying conditions such as microwave drying, hot air drying , oven drying modes should better study.
- Further research on sedimentation analysis should be carried out on the yield of rosemary essential oil so that, approximate weight of the oil and water can be determined.
- Further study should be conducted on the quality of the rosemary essential oil obtained at each operating conditions using GC-MS. This will provide alternative to manufacturers on the minimum conditions required to get a specific quality of rosemary essential oil.
- Further detail research on feasibility studies are required before implementing this research result.

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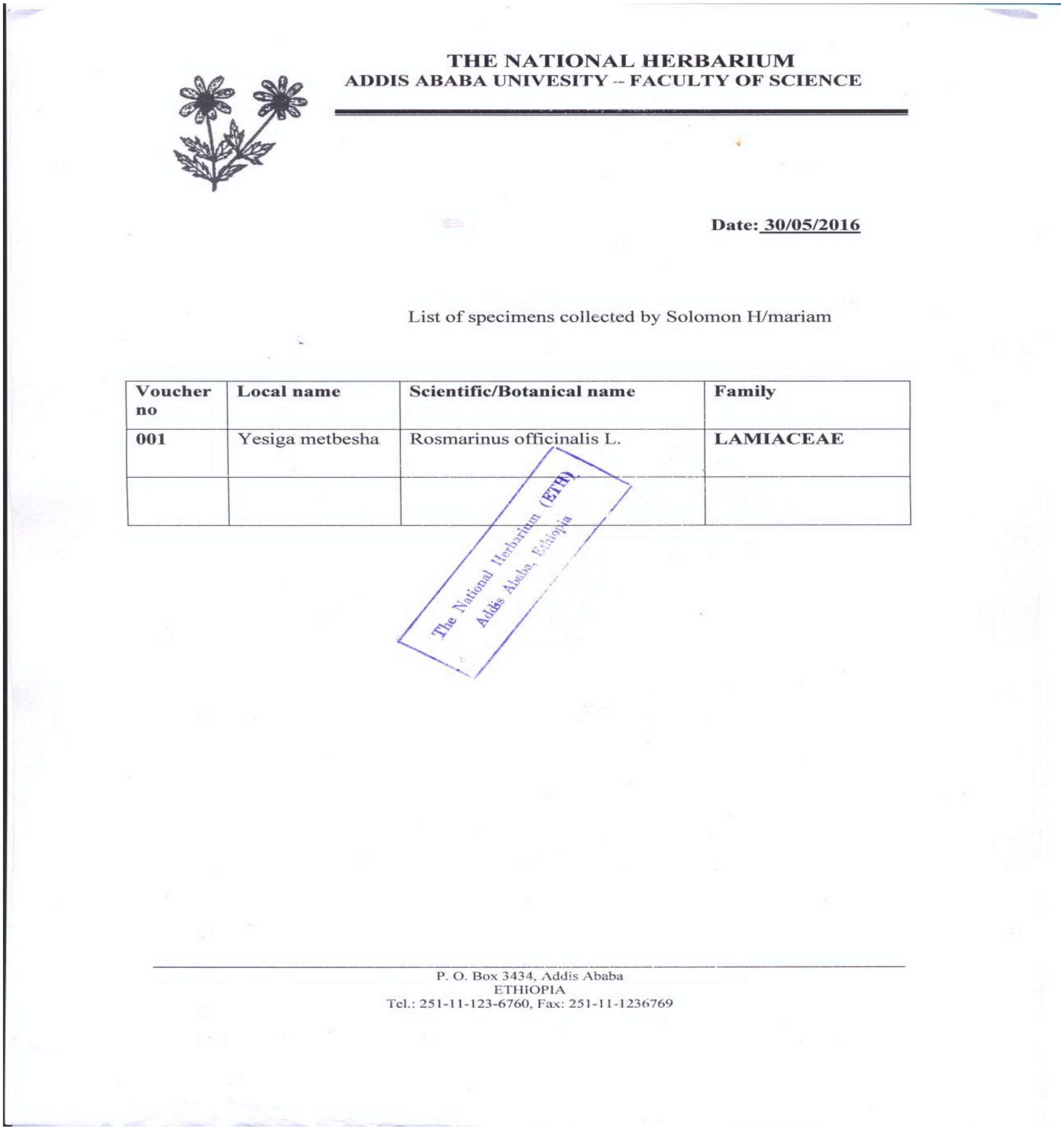
APPENDICES

Appendix A: Experimental Run Data Analysis



THE NATIONAL HERBARIUM
ADDIS ABABA UNIVERSITY -- FACULTY OF SCIENCE

Date: 30/05/2016

List of specimens collected by Solomon H/mariam

Voucher no	Local name	Scientific/Botanical name	Family
001	Yesiga metbesha	Rosmarinus officinalis L.	LAMIACEAE

The National Herbarium (ETH)
Addis Ababa, Ethiopia

P. O. Box 3434, Addis Ababa
ETHIOPIA
Tel.: 251-11-123-6760, Fax: 251-11-1236769

Figure A1: Species of rosemary plant leaves

A 1: Quantitative Analysis Result

Table A1: Mass of rosemary essential oil

Factors		**Yield of essential oil (g)**			
Extraction Time (min)	Drying condition	Replicates			
		1	2	3	Average
30	Fresh	3.2	4.5	3.6	3.78
	Shade dried	6.2	6.5	7.5	6.73
	Sun dried	10.8	8.4	8.6	9.27
60	Fresh	4.6	6.1	5.5	5.4
	Shade dried	6.9	7.8	8.6	7.77
	Sun dried	11.4	8.8	10.2	10.13
120	Fresh	4.1	5.2	5.6	4.97
	Shade dried	8.6	9.9	7.9	8.8
	Sun dried	9.3	13.2	11.6	11.37
150	Fresh	4.1	5.2	5.6	4.97
	Shade dried	8.6	9.9	7.9	8.8
	Sun dried	9.3	13.2	11.6	11.37

A2: Qualitative analysis of rosemary essential oil

Data File name	00401004.D	**Sample Name**	ROS-1
Sample Type		**Position**	4
Instrument Name	AAU	**User Name**	Estif
Acq Method	Essential oil.M	**Acquired Time**	6/3/2016 2:45:42 PM
IRM Calibration Status	Not Applicable	**DA Method**	default.m
Comment			

Expected Barcode		**Sample Amount**	
Dual Inj Vol	1	**Tune Name**	ATUNE.U
Tune Path	D:\MassHunter\GCMS\1\5977\	**MSFirmware Version**	6.00.21
Acquisition Time #2	2016-06-03 11:45:42Z	**Operator Name**	Estif
Run Completed Flag	True	**Acquisition SW Version**	Mass Hunter GC/MS Acquisition B.07.02.1938 08-Sep-2014 Copyright © 1989-2014 Agilent Technologies, Inc.

Fragment or Voltage		**Collision Energy**	0	**Ionization Mode**	Unspecified

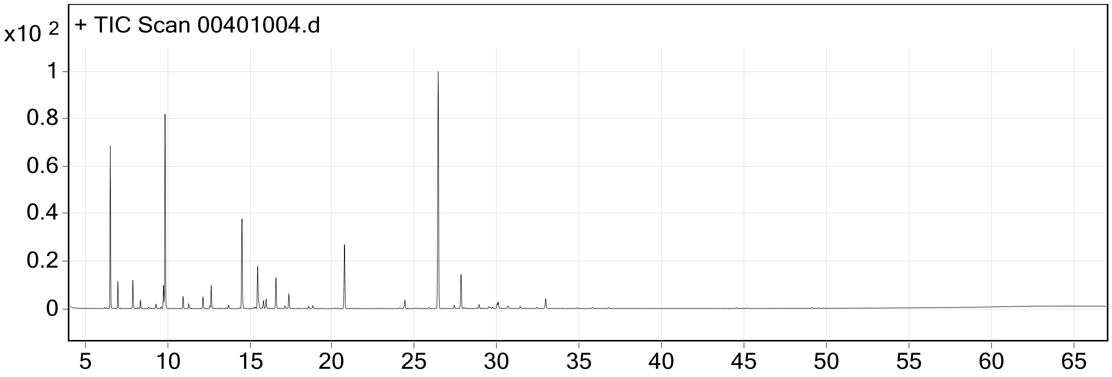


Figure A2: User chromatogram of sun dried rosemary leaves

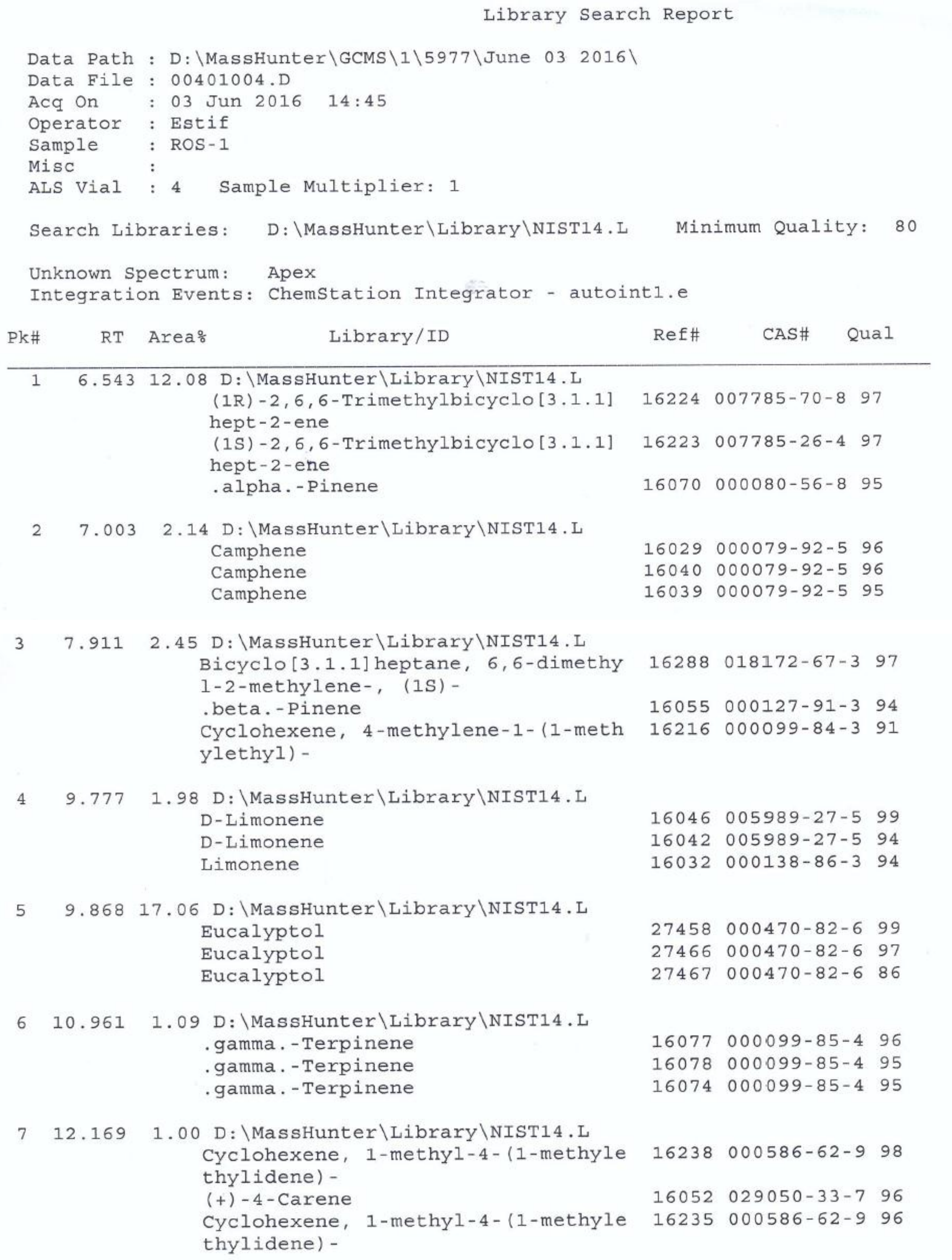
TableA2: Integration peak list of sun dried rosemary essential oil

Peak	Start	RT	End	Height	Area	Area %
1	6.455	6.536	6.684	33100290.23	82429577.08	43.94
2	6.913	6.994	7.115	5529304.12	14608481.2	7.79
3	7.828	7.909	8.052	5755495.33	16707860.56	8.91

Table A2: Cont'd

4	8.314	8.366	8.457	1561445.49	4160736.68	2.22
5	9.245	9.308	9.398	902213.96	2602630.79	1.39
6	9.7	9.766	9.806	4676031.69	13654283.59	7.28
7	9.806	9.86	10.021	39575955.71	116313615.7	62
8	10.883	10.95	11.031	2451137.14	7426737.17	3.96
9	11.228	11.3	11.475	941642.54	2874419.86	1.53
10	12.095	12.161	12.279	2216474.43	6772362.63	3.61
11	12.605	12.659	12.766	4793958.78	14832003.36	7.91
12	13.641	13.708	13.813	689577.26	2450974.64	1.31
13	14.449	14.529	14.65	18231748.44	62749533.53	33.45
14	15.39	15.485	15.66	8667651.06	34579461.39	18.43
15	15.767	15.835	15.902	1565385.78	5567177.6	2.97
16	15.902	15.996	16.117	1897200.08	6600348.36	3.52
17	16.507	16.588	16.763	6275699.27	21184227.83	11.29
18	17.064	17.14	17.274	531345.59	1972946.19	1.05
19	17.274	17.369	17.553	2926720.73	10631293.37	5.67
20	18.74	18.835	18.983	577139.87	2458532.15	1.31
21	20.652	20.746	20.908	13025317.93	46457599.54	24.76
22	24.289	24.406	24.554	1659756.86	6833059.01	3.64
23	26.317	26.438	26.668	48220604.27	187600800.4	100
24	27.34	27.407	27.515	628302.55	2325983.7	1.24
25	27.716	27.824	27.932	6912976.12	25967457.16	13.84
26	28.847	28.928	29.089	775454.23	3004030.33	1.6
27	29.435	29.52	29.668	385876.82	2486312.26	1.33
28	29.937	30.085	30.166	1312051.91	7810797.73	4.16
29	30.564	30.677	30.793	481416.24	2373071.72	1.26
30	32.843	32.964	33.126	1961565.32	8325862.25	4.44

Table A3: Library search report of GC-MS for sun dried rosemary essential oil.



```
                                        Library Search Report

 Data Path : D:\MassHunter\GCMS\1\5977\June 03 2016\
 Data File : 00401004.D
 Acq On    : 03 Jun 2016  14:45
 Operator  : Estif
 Sample    : ROS-1
 Misc      :
 ALS Vial  : 4   Sample Multiplier: 1

 Search Libraries:   D:\MassHunter\Library\NIST14.L    Minimum Quality:  80

 Unknown Spectrum:   Apex
 Integration Events: ChemStation Integrator - autoint1.e

Pk#      RT  Area%          Library/ID                      Ref#      CAS#    Qual
______________________________________________________________________________________

  1   6.543 12.08 D:\MassHunter\Library\NIST14.L
                 (1R)-2,6,6-Trimethylbicyclo[3.1.1]   16224 007785-70-8 97
                 hept-2-ene
                 (1S)-2,6,6-Trimethylbicyclo[3.1.1]   16223 007785-26-4 97
                 hept-2-ene
                 .alpha.-Pinene                       16070 000080-56-8 95

  2   7.003  2.14 D:\MassHunter\Library\NIST14.L
                 Camphene                             16029 000079-92-5 96
                 Camphene                             16040 000079-92-5 96
                 Camphene                             16039 000079-92-5 95

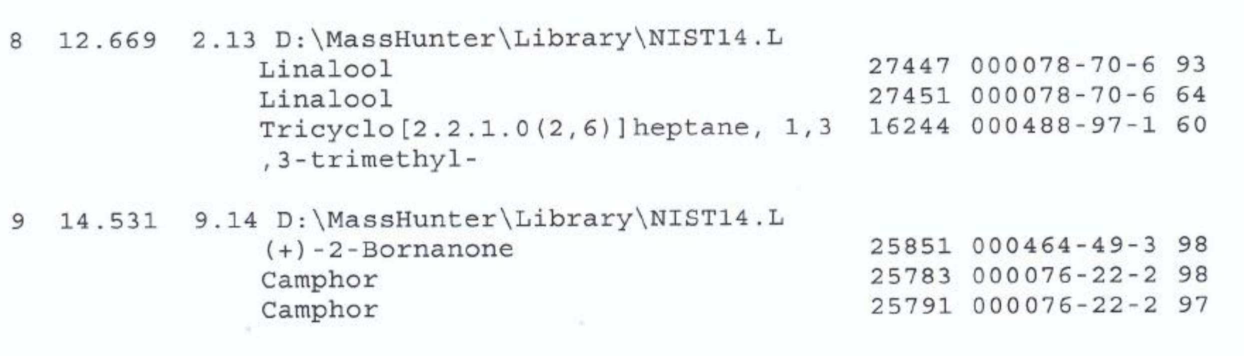
 3    7.911  2.45 D:\MassHunter\Library\NIST14.L
                 Bicyclo[3.1.1]heptane, 6,6-dimethy   16288 018172-67-3 97
                 l-2-methylene-, (1S)-
                 .beta.-Pinene                        16055 000127-91-3 94
                 Cyclohexene, 4-methylene-1-(1-meth   16216 000099-84-3 91
                 ylethyl)-

 4    9.777  1.98 D:\MassHunter\Library\NIST14.L
                 D-Limonene                           16046 005989-27-5 99
                 D-Limonene                           16042 005989-27-5 94
                 Limonene                             16032 000138-86-3 94

 5    9.868 17.06 D:\MassHunter\Library\NIST14.L
                 Eucalyptol                           27458 000470-82-6 99
                 Eucalyptol                           27466 000470-82-6 97
                 Eucalyptol                           27467 000470-82-6 86

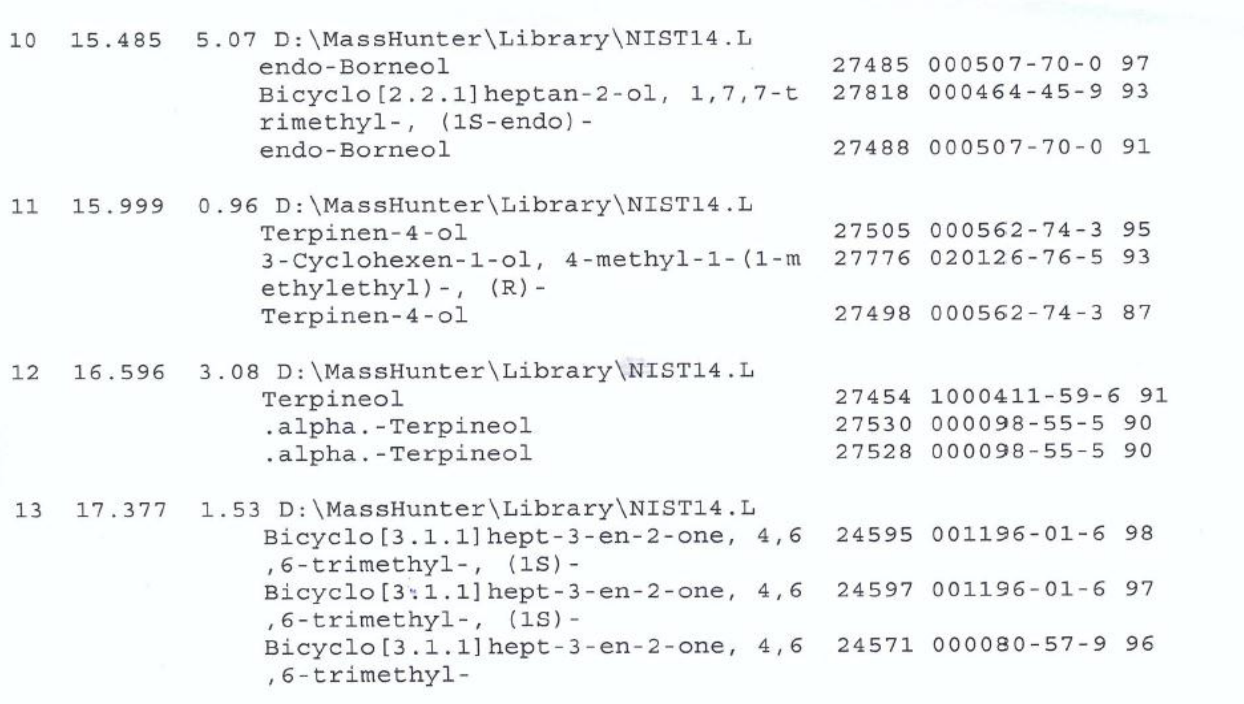
 6   10.961  1.09 D:\MassHunter\Library\NIST14.L
                 .gamma.-Terpinene                    16077 000099-85-4 96
                 .gamma.-Terpinene                    16078 000099-85-4 95
                 .gamma.-Terpinene                    16074 000099-85-4 95

 7   12.169  1.00 D:\MassHunter\Library\NIST14.L
                 Cyclohexene, 1-methyl-4-(1-methyle   16238 000586-62-9 98
                 thylidene)-
                 (+)-4-Carene                         16052 029050-33-7 96
                 Cyclohexene, 1-methyl-4-(1-methyle   16235 000586-62-9 96
                 thylidene)-
```



```
8   12.669   2.13 D:\MassHunter\Library\NIST14.L
                  Linalool                                27447 000078-70-6 93
                  Linalool                                27451 000078-70-6 64
                  Tricyclo[2.2.1.0(2,6)]heptane, 1,3      16244 000488-97-1 60
                  ,3-trimethyl-

9   14.531   9.14 D:\MassHunter\Library\NIST14.L
                  (+)-2-Bornanone                         25851 000464-49-3 98
                  Camphor                                 25783 000076-22-2 98
                  Camphor                                 25791 000076-22-2 97
```

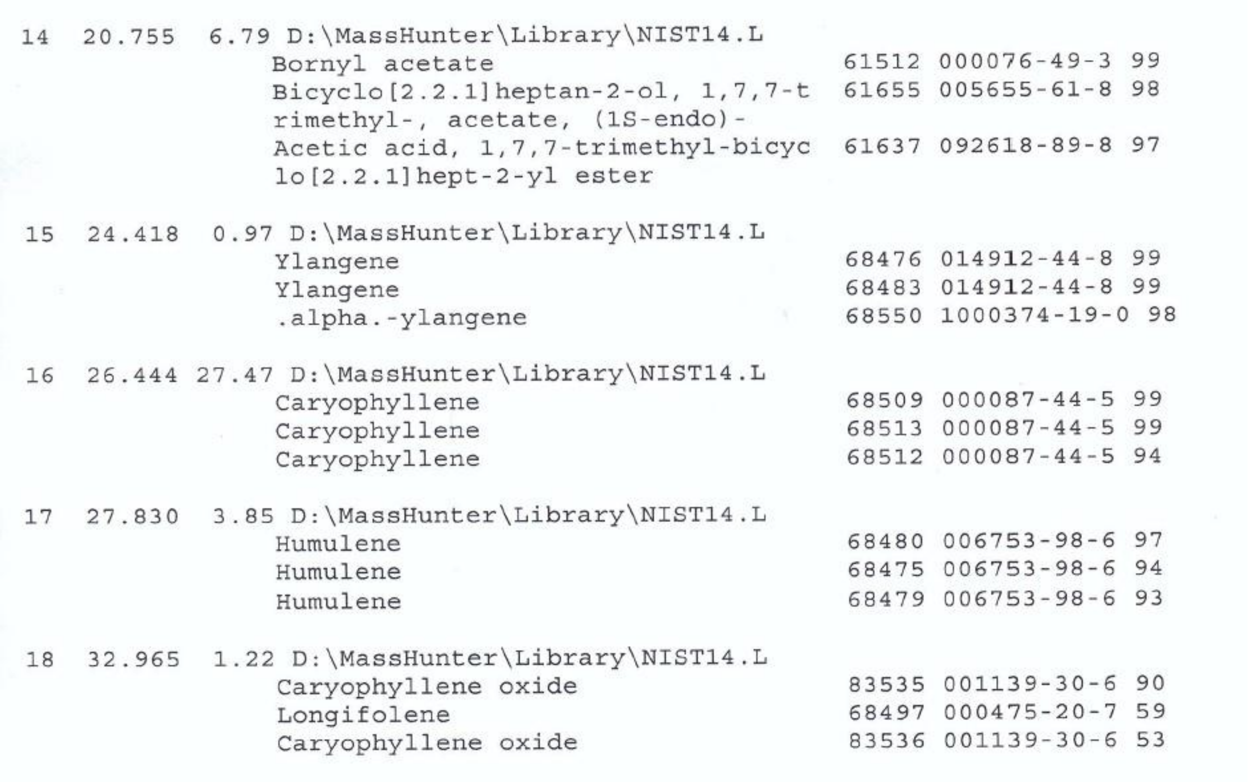


```
10  15.485   5.07 D:\MassHunter\Library\NIST14.L
                  endo-Borneol                          27485 000507-70-0 97
                  Bicyclo[2.2.1]heptan-2-ol, 1,7,7-t    27818 000464-45-9 93
                  rimethyl-, (1S-endo)-
                  endo-Borneol                          27488 000507-70-0 91

11  15.999   0.96 D:\MassHunter\Library\NIST14.L
                  Terpinen-4-ol                         27505 000562-74-3 95
                  3-Cyclohexen-1-ol, 4-methyl-1-(1-m    27776 020126-76-5 93
                  ethylethyl)-, (R)-
                  Terpinen-4-ol                         27498 000562-74-3 87

12  16.596   3.08 D:\MassHunter\Library\NIST14.L
                  Terpineol                             27454 1000411-59-6 91
                  .alpha.-Terpineol                     27530 000098-55-5 90
                  .alpha.-Terpineol                     27528 000098-55-5 90

13  17.377   1.53 D:\MassHunter\Library\NIST14.L
                  Bicyclo[3.1.1]hept-3-en-2-one, 4,6    24595 001196-01-6 98
                  ,6-trimethyl-, (1S)-
                  Bicyclo[3.1.1]hept-3-en-2-one, 4,6    24597 001196-01-6 97
                  ,6-trimethyl-, (1S)-
                  Bicyclo[3.1.1]hept-3-en-2-one, 4,6    24571 000080-57-9 96
                  ,6-trimethyl-
```



```
14  20.755   6.79 D:\MassHunter\Library\NIST14.L
                  Bornyl acetate                        61512 000076-49-3 99
                  Bicyclo[2.2.1]heptan-2-ol, 1,7,7-t    61655 005655-61-8 98
                  rimethyl-, acetate, (1S-endo)-
                  Acetic acid, 1,7,7-trimethyl-bicyc    61637 092618-89-8 97
                  lo[2.2.1]hept-2-yl ester

15  24.418   0.97 D:\MassHunter\Library\NIST14.L
                  Ylangene                              68476 014912-44-8 99
                  Ylangene                              68483 014912-44-8 99
                  .alpha.-ylangene                      68550 1000374-19-0 98

16  26.444 27.47 D:\MassHunter\Library\NIST14.L
                  Caryophyllene                         68509 000087-44-5 99
                  Caryophyllene                         68513 000087-44-5 99
                  Caryophyllene                         68512 000087-44-5 94

17  27.830   3.85 D:\MassHunter\Library\NIST14.L
                  Humulene                              68480 006753-98-6 97
                  Humulene                              68475 006753-98-6 94
                  Humulene                              68479 006753-98-6 93

18  32.965   1.22 D:\MassHunter\Library\NIST14.L
                  Caryophyllene oxide                   83535 001139-30-6 90
                  Longifolene                           68497 000475-20-7 59
                  Caryophyllene oxide                   83536 001139-30-6 53
```

Appendix B: Design Expert Statistical Analysis Result of Rosemary Essential Oil

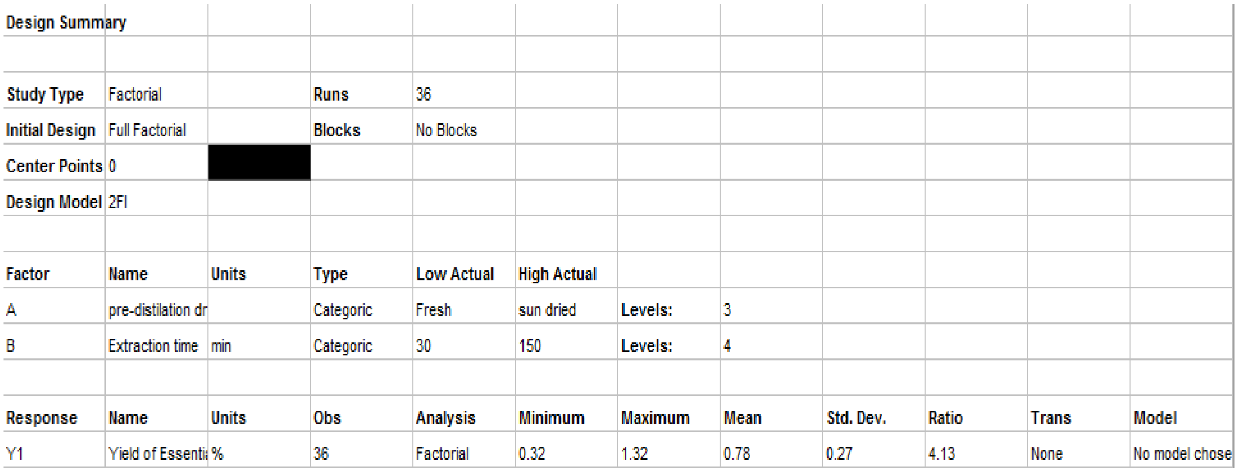
Table B1: Analysis of experimental (actual) data of rosemary essential oil

Standard order	Run order	Blocks	Factor 1 A: pre-distillation drying	Factor 2 B: extraction time(min)	Response 1 Yield of essential oil (%)
1	8	Block 1	Fresh	30	0.32
2	27	Block 1	Fresh	30	0.45
3	29	Block 1	Fresh	30	0.36
4	9	Block 1	shade dried	30	0.62
5	24	Block 1	shade dried	30	0.65
6	10	Block 1	shade dried	30	0.75
7	30	Block 1	sun dried	30	1.08
8	2	Block 1	sun dried	30	0.84
9	25	Block 1	sun dried	30	0.86
10	12	Block 1	Fresh	60	0.46
11	11	Block 1	Fresh	60	0.61
12	20	Block 1	Fresh	60	0.55
13	26	Block 1	shade dried	60	0.69
14	22	Block 1	shade dried	60	0.78
15	28	Block 1	shade dried	60	0.86
16	31	Block 1	sun dried	60	1.14
17	21	Block 1	sun dried	60	0.88
18	18	Block 1	sun dried	60	1.02
19	15	Block 1	Fresh	120	0.41
20	33	Block 1	Fresh	120	0.52
21	34	Block 1	Fresh	120	0.56
22	6	Block 1	shade dried	120	0.86

Table B1: Cont’d

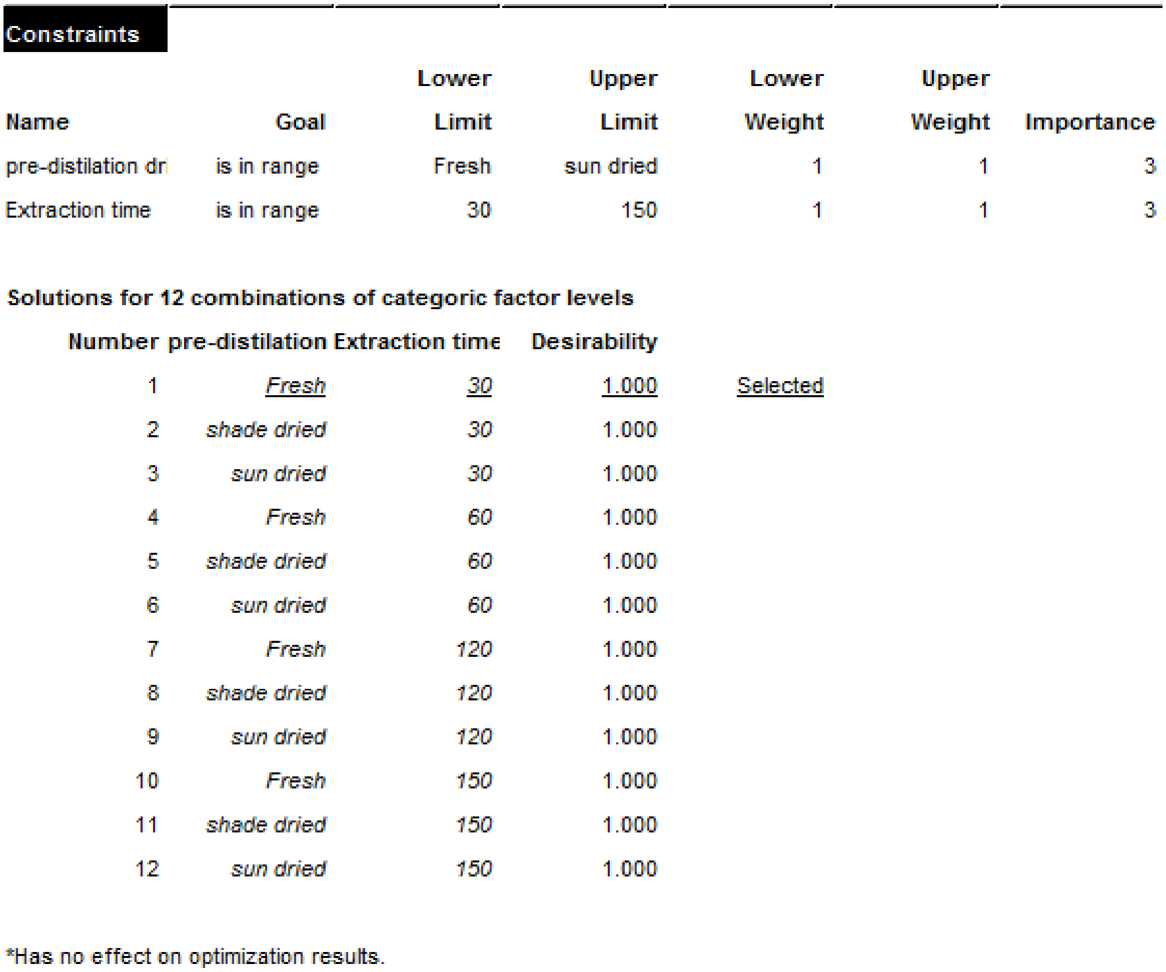
23	17	Block 1	shade dried	120	0.99
24	13	Block 1	shade dried	120	0.79
25	1	Block 1	sun dried	120	0.93
26	32	Block 1	sun dried	120	1.32
27	7	Block 1	sun dried	120	1.16
28	36	Block 1	Fresh	150	0.41
29	16	Block 1	Fresh	150	0.52
30	35	Block 1	Fresh	150	0.56
31	3	Block 1	shade dried	150	0.86
32	4	Block 1	shade dried	150	0.99
33	5	Block 1	shade dried	150	0.79
34	19	Block 1	sun dried	150	0.93
35	14	Block 1	sun dried	150	1.32
36	23	Block 1	sun dried	150	1.16

Table B2: Design summery of rosemary essential oil



Design Summary											
Study Type	Factorial		**Runs**	36							
Initial Design	Full Factorial		**Blocks**	No Blocks							
Center Points	0	[redacted]									
Design Model	2FI										
Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**						
A	pre-distilation dr		Categoric	Fresh	sun dried	**Levels:**	3				
B	Extraction time	min	Categoric	30	150	**Levels:**	4				
Response	**Name**	**Units**	**Obs**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
Y1	Yield of Essenti	%	36	Factorial	0.32	1.32	0.78	0.27	4.13	None	No model chose

Table B3: Constraints and desirability of yield of rosemary essential oil



Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
pre-distilation dr	is in range	Fresh	sun dried	1	1	3
Extraction time	is in range	30	150	1	1	3

Solutions for 12 combinations of categoric factor levels

Number	pre-distilation	Extraction time	Desirability	
1	*Fresh*	*30*	1.000	Selected
2	*shade dried*	*30*	1.000	
3	*sun dried*	*30*	1.000	
4	*Fresh*	*60*	1.000	
5	*shade dried*	*60*	1.000	
6	*sun dried*	*60*	1.000	
7	*Fresh*	*120*	1.000	
8	*shade dried*	*120*	1.000	
9	*sun dried*	*120*	1.000	
10	*Fresh*	*150*	1.000	
11	*shade dried*	*150*	1.000	
12	*sun dried*	*150*	1.000	

*Has no effect on optimization results.

Table B4: Design matrix valuation for response surface cubic model

Degrees of Freedom for Evaluation

Model	9
Residuals	26
Lack Of Fit	*2*
Pure Error	*24*
Corr Total	35

A recommendation is a minimum of 3 lack of fit df and 4 df for pure error.

This ensures a valid lack of fit test.

Fewer df will lead to a test that may not detect lack of fit.

				Power at 5 % alpha level for effect of		
Term	**StdErr****	**VIF**	**Ri-Squared**	**0.5 Std. Dev.**	**1 Std. Dev.**	**2 Std. Dev.**
A[1]	0.46			16.3 %	53.2 %	99.0 %
A[2]	0.46					
B	0.63	9.03	0.8892	20.8 %	62.7 %	99.5 %
A[1]B	0.30			11.8 %	35.4 %	91.5 %
A[2]B	0.30					
B^2	0.44	1.00	0.0000	19.2 %	58.2 %	99.1 %
$A[1]B^2$	0.63			6.4 %	11.1 %	32.2 %
$A[2]B^2$	0.63					
B^3	0.70	9.03	0.8892	6.4 %	10.5 %	27.8 %

****Basis Std. Dev. = 1.0**

For Categorical Terms, The minimum Power for each group of terms is reported.

12 Solutions found

Number of Starting Points: 12 for 12 combinations of categoric factor levels

pre-distilation	**Extraction time**
Fresh	30
shade dried	30
sun dried	30
Fresh	60
shade dried	60
sun dried	60
Fresh	120
shade dried	120
sun dried	120
Fresh	150
shade dried	150
sun dried	150

Table B5: Model adequacy measure of rosemary essential oil

Std. Dev.	0.114812	R-Squared	0.866124
Mean	0.777778	Adj R-Squared	0.819782
C.V. %	14.76154	Pred R-Squared	0.742547
PRESS	0.659084	Adeq Precision	12.466

	Coefficient		Standard	95% CI	95% CI	
Term	Estimate	Df	Error	Low	High	VIF
Intercept	0.826852	1	0.037192	0.750402	0.903302	
A[1]	-0.2813	1	0.052598	-0.38941	-0.17318	
A[2]	0.018704	1	0.052598	-0.08941	0.12682	
B-Extraction time	0.051667	1	0.072725	-0.09782	0.201156	9.027778
A[1]B	-0.04444	1	0.03423	-0.11481	0.025917	
A[2]B	0.019556	1	0.03423	-0.05081	0.089917	
B^2	-0.07852	1	0.051028	-0.18341	0.02637	1
A[1]B^2	-0.03037	1	0.072164	-0.17871	0.117965	
A[2]B^2	0.00963	1	0.072164	-0.13871	0.157965	
B^3	0.037778	1	0.080682	-0.12807	0.203621	9.027778

Table B6: Sequential Model Sum of Squares [Type I] for yield of rosemary essential oil

Source	Sum of squares	df	Mean Square	F-Value	P- value Prob > F	
Mean vs Total	21.77778	1	21.77778			
Linear vs Mean	2.158427	3	0.719476	57.32936	< 0.0001	Suggested
2FI vs Linear	0.022329	2	0.011164	0.883108	0.4240	
Quadratic vs 2FI	0.031211	1	0.031211	2.600511	0.1177	
Cubic vs Quadratic	0.005329	3	0.001776	0.134754	0.9384	
Residual	0.342727	26	0.013182			
Total	24.3378	36	0.67605			

"Sequential Model Sum of Squares [Type I]": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Table B7: Lack of fit test for rosemary essential oil

Source	Sum of Squares	df	Mean Square	F-Value	P-value Prob > F	
Linear	0.069196	8	0.008649	0.624509	0.7491	Suggested
2FI	0.046867	6	0.007811	0.563979	0.7546	
Quadratic	0.015656	5	0.003131	0.226073	0.9476	
Cubic	0.010327	2	0.005163	0.372804	0.6927	

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Table B8: Model summery statistics of rosemary essential oil

Source	Std Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.112026	0.843128	0.828421	0.801711	0.507623	Suggested
2FI	0.112438	0.85185	0.827159	0.785303	0.54963	
Quadratic	0.109553	0.864042	0.835913	0.789108	0.539889	
Cubic	0.114812	0.866124	0.819782	0.742547	0.659084	

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Appendix C: Laboratory Equipments and Samples Photos.

Figure C1:   Rosemary plant (*Rosmarinus officinalis*)

Figure C2a: Fresh rosemary leaves.

Figure C2$_b$: Shade dried rosemary leaves

Figure C2c: Sun dried rosemary leaves

Figure C3: Steam distillation chamber

Figure C4: Gravity oil separator with pure essential oil and hydrosol

Figure C5. Pure rosemary essential oil samples

Figure C6. Vibro-viscometer

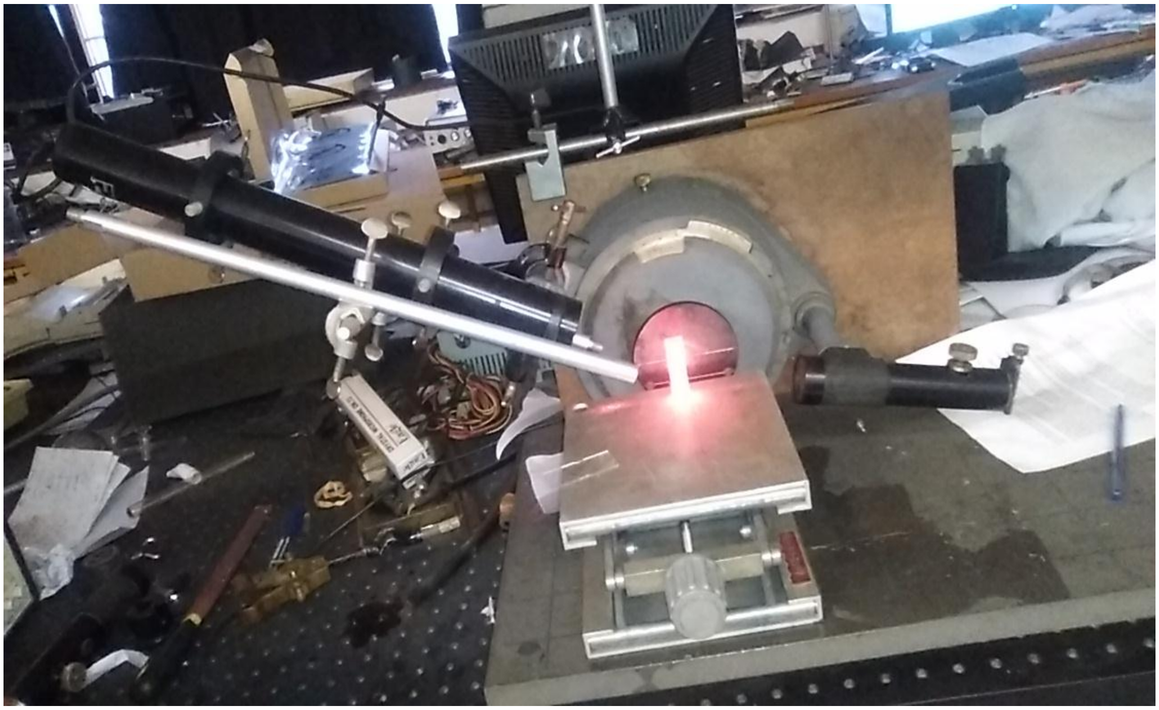


Figure C7: Determination of refractive index by polarimeter

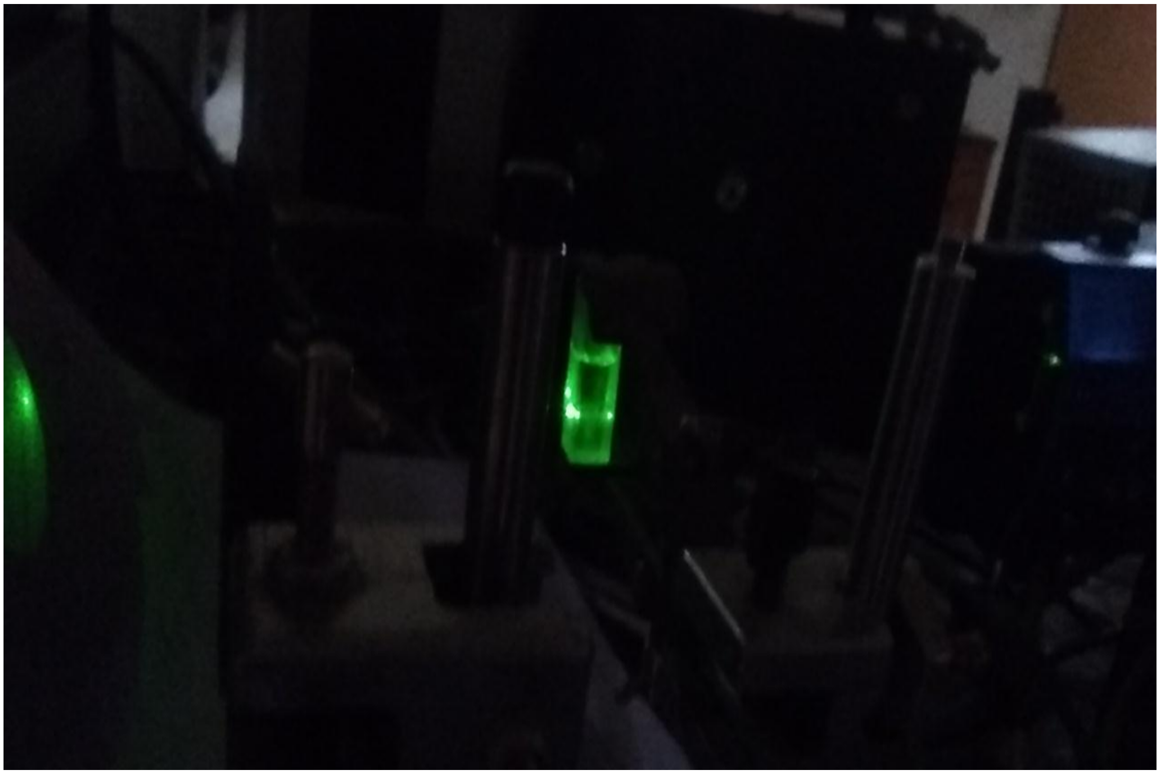


Figure C8: Determination of optical rotation by polarimeter

Figure C9: Gas Chromatography Mass Spectroscopy

Figure C10. Pure rosemary essential oil inside gravity separator.

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Solomon Hailemariam

Signature: ______________________

Place: Addis Ababa, Ethiopia

Date of submission: ______________________

This thesis has been submitted for examination with my approval as University advisor.

Name: Eng. Teshome Worku (Assistant Prof.)
Signature: ______________________