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Production, optimization and characterization of essential oil from spearmint leaves

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This is to certify that the thesis prepared by Gebremichael Gebrehiwot, entitled: “Production, Optimization and Characterization of essential Oil from spearmint leaves” and submitted in partial fulfillment of the requirements for Degree of Master of Sciences in Process Engineering that complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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DECLARATION

I, the undersigned, declare that this thesis entitled "Production, Optimization and Characterization of essential oil from spearmint leaves" is my original work. The matter embodied in this thesis has not been submitted by any other person for an award of degree in this or any other university and all resource of information used or quoted in this thesis have been duly acknowledged.

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ABSTRACT

The national consumption of essential oils in Ethiopia is increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The objective of this study was the production, optimization and characterization of oil from spearmint leaves using hydro distillation method. The effect of three factors, drying methods (shade, sun and oven drying), drying duration (0, 3 and 7 days) and distillation time (1, 1.5 and 2 hours) on the oil yield was investigated. This paper includes the physical properties of spearmint oil like, density (0.915), refractive index (1.43), pH (6.06) and dynamic viscosity (0.47mPaS).And the chemical properties; saponification value (41.83), acid value (0.045), peroxide value (0.67) and iodine value (17.8) were determined. Design expert software was used to optimize the oil yield and examine the effect of the three factors. General factorial design was chosen for the above mentioned optimization as well as for the data analysis (ANOVA).Then from this study, the best operating conditions are found as drying method (shade), drying time (7 days) and a distillation time of 2 hours. At this condition, a maximum yield of 2.27 % oil is obtained. This research also determined the chemical composition of the oil. From GC/MS result 41 components were obtained.

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List of Acronyms

AV	Acid value
EFSA	European Food Safety Authority
FD	Freeze Drying
GC/MS	Gas Chromatography Mass spectrometry
HAD	Hot Air drying
HP	High Pressure
IV	Iodine Value
LP	low Pressure
PV	Peroxide Value
SV	Saponification Value

1. INTRODUCTION

1.1 Back ground

Essential oils by definition are the liquid products of steam distillation of plant parts. An essential oil may contain tens or hundreds of volatile and non-volatile compounds, the cause of their characteristic fragrance or flavor. Essential oil are present in plants in specialized cells/glands (subcuticular spaces of glandular cells, organelles.), these glands may be at anywhere on plant body depending upon the morphology and physiology of the plant. It may be on leaves, flowers, stems, roots, bark or wood.

Essential oils are plant-based volatile oils with strong aromatic components that are made up of different chemical compounds. For example, alcohols, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components of essential oil (Rassem, 2016).pure essential oils are derived from different parts of the plants. These essential oils have commercial value due to their properties. They are widely used in various fields of industries like perfumery industries and pharmaceuticals.

The use and processing of essential oils began in the East more than 2500 years ago. The process of distillation, which is the technical basis of the essential oil industry, was also conceived and first employed in the Orient, especially in Egypt, Persia and India (Hernandez, 2000).

The national production and consumption of essential oils and perfumes in Ethiopia are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies that suit to essential oil processing are of great significance and are still being used in many parts of the globe.

Advanced processing methods, based upon modern principles of plant breeding, mechanized agriculture, engineering and mass production, represent the competing counterpart of the primitive methods described above. The oils obtained in regular essential oil factories generally possess a quality superior to those produced by natives in backward districts; but the operating expenses are high. In addition to the higher standard of living and consequent higher wages and salaries involved, the amortization of invested capital, taxes and other general overhead expenses increase the costs of production (GUENTHER, 1948).

One of the major important essential oil herbs is mint plant which exist widely in Mediterranean region and in small scale home gardens and agricultural research areas in Ethiopia, species by the name of spearmint (*Mentha spicata), and local (WGSM_03*) (MENGESHA, 2015). Spearmint and peppermint grow best in wet or moist, slightly acidic soils. They tolerate light conditions ranging from full sunlight to partial shade. (Salim, 2016).

1.2 Statement of the problem

Now adays many essential oils requiring firms are getting introduced, right from the cosmetics and soap factories to different food spices. Almost all the required essential oils for the soap factories are imported from abroad, from India, Germany, Ireland, Italy, South Africa, Great Britain and China (Ethiopian Customs and Revenue Authority various years).This import of essential oils adds the cost of production of the soap factories. The very reason to the initiation of this spearmint oil production research is the currently high cost (750 birr/kg from Yegna chemicals) of scenting soap additives and the herb being nice aromatic and easily available but yet unutilized. Furthermore no studies are yet conducted in Ethiopia on characterization of spearmint oils and hence it is believed this spearmint oil research will contribute to the essential oils of its kind.

1.3 OBJECTIVES

1.3.1 General objective

The general objective of the study was to extract and optimize essential oil from spearmint leaves and study the chemical constituents.

1.3.2 Specific objectives

The study had the following specific goals

- Characterization of raw material
- Optimization of process variables for maximization of oil yield
- Chemical analysis of the produced oil

1.4 Significance of the study

The study has the following importance

- Broadens the investigation of aromatic herbs and their chemical characterization that had been locally seen to be insignificant
- The study suggested an alternative scenting additives to the small scale liquid soap factories and can show a new product line to the existing large soap factories
- It provides a starting ground to further researches on spearmint oils

1.5 Scope of the study

The research covered the extraction of the spearmint oil by hydro distillation and characterizing its different chemical and physical properties. It also discovered the constituent compounds by GC-MS. The oil yield was optimized considering three factors.

2. LITERATURE REVIEW

2.1- Essential Oils

The essential oil industry in its present stage in addition to the production and distribution of essential oils and the improvement of methods, or to the establishment and maintenance of standards of quality has come more and more to be concerned with the development, production and testing of synthetic aromatics and mixtures which today find their way into so many products of our advanced civilization (GUENTHER, 1948).

Essential oils are extracts from fragrant plants that are used extensively in the perfume and flavoring industry. Most components of essential oils are terpenes that contain multiples of a five carbon structural unit, the isoprene unit.

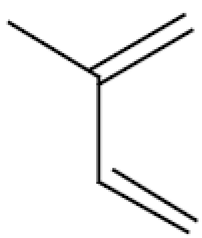


Fig 2.1 Isoprene unit

Essential oils are concentrated volatile aromatic compounds produced by plants the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant. Essential oils are frequently referred to as the “life force” of plants. (Salih, 2011).Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material is required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics.

The mint oils as a group {peppermint, spearmint (both grown in the USA) and corn mint (produced in India and China and as a by-product of menthol production)} are the highest value oils, with peppermint selling for about $12 -15 a pound (Hernandez, 2000).

Mentha, commonly called mint, is a genus in the Lamiaceae. The genus consists of 25–30 species (Ali, 2002). Many varieties of mint exist and the cultivars selected for commercial production are generally specific to a geographic area. In the eastern hemisphere the most common species grown and studied is M. arvensis (Bahl, 2000). Mentha arvensis is commonly called field mint, wild mint, corn mint, or Japanese mint. Mentha spicata, spearmint, and peppermint are the most common species cultivated in the western hemisphere (Ullah, 2012). Spearmint and peppermint are economic crops that are used raw or processed into oil for a variety of consumption purposes.

Usually, essential oils take the name of the plant species from which it is derived, e.g., rose oil, peppermint oil etc. this implies that each type of essential oil of a given plant is different from the others. So oil from particular plant species or part is composed of particular chemical compounds or active constitutes which tends the oil to exhibit a peculiar fragrance and other physicochemical characteristics. The type and extent of the active constitute present in a given oil usually determine the physicochemical property of that oil, which ultimately determine the use and application of that oil. As we mentioned above the, the ultimate sources of essential oils are aromatic plant species. And the oil from spearmint leaves is called spearmint oil (http://www.eiar.gov.et/).

2.2 - Spearmint Oil

Mint plants are important essential oil plants that can be successfully cultivated in Ethiopia, especially the common spearmint (*Mentha spicata* L.). Spearmint species belong to the family Labiate (Lamiaceae), which is considered as a famous medicinal and aromatic family (Guenther, 1975)Spearmint is a perennial herb known as “Nanaa”. It is shown in the figure below

B) dry

A)Fresh

fig 2.2 spearmint leaves

2.3 Extraction of spearmint Oils

The choice of a particular process for the extraction of essential oil is generally dictated by the following considerations:

a) Sensitivity of the essential oil to the action of heat and water.

b) Volatility of the essential oil.

c) Water solubility of the essential oil.

Distillation is the most popular, widely used and cost-effective method for producing essential oils throughout the world. Distillation of aromatic plants simply consists of vaporizing or liberating the oils from the plant cellular membranes in the presence of moisture, by applying high temperature and then cooling the vapor mixture to separate the oil from the water on the basis of the immiscibility and density of the essential oil with respect to water.

After spearmint is harvested, distillation is used to obtain the essential oil. Mint distillation happens in the mint fields. The cut spearmint plants are transferred to a large metal distillation tub on the back of a truck. When the tub is full, it is fitted with a steam-tight lid containing an outlet pipe. Steam from a boiler is then piped into the bottom of the distillation tub. Steam condenses on the surface of the spearmint and causes the spearmint oil to vaporize. The vaporized spearmint oil is passed through the outlet pipe on the top of the tub and sent to a condenser, where the oil is cooled back to a liquid. The spearmint oil can be easily separated from the remaining water, as the two liquids are immiscible (Lawrence, 2007)

2.3.1 Hydro distillation

In order to isolate essential oils by hydro distillation, the aromatic plant material is packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the essential oil is freed from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water.

Essential oils with high solubility in water and those that are susceptible to damage by heat cannot be steam distilled. Also, the oil must be steam volatile for steam distillation to be feasible. Most of the essential oils in commerce are steam volatile, reasonably stable to heat and practically insoluble in water; hence they are suitable for processing by steam distillation.

Essential oils are a mixture of various aroma chemicals, basically monoterpenes, sesquiterpenes and their oxygenated derivatives, having a boiling point ranging from 150° to 300° C. When the plant material is subjected to heat in the presence of moisture from the steam, these oils are liberated from the plant. For the oil to change from the liquid to the vapor phase, it must receive latent heat that, within the tank, can only come from condensing steam. Consequently, the temperature of the steam within the still must be higher than the temperature at which the oil boils in the presence of water on the surface of the plant material; otherwise there would not be a temperature gradient to take the latent heat from the condensing steam to vaporize the oil droplet. Thus, the energy from the steam in form of heat as latent heat of vaporization converts the oil into a vapor. But, as the boiling point of the oil is higher than that of water, the vaporization takes place with steam on the basis of their relative vapor pressures.

Advantages of hydro-distillation

During water distillation, all parts of the plant charge must be kept in motion by boiling water; this is possible when the distillation material is charged loosely and remains loose in the boiling water. For this reason only, water distillation possesses one distinct advantage, i.e. that it permits processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. Other practical advantages of water distillation are that the stills are inexpensive, easy to construct and suitable for field operation. These are still widely used with portable equipment in many countries.

The main disadvantage of water distillation is that complete extraction is not possible. Besides, certain esters are partly hydrolyzed and sensitive substances like aldehydes tend to polymerize. Water distillation requires a greater number of stills, more space and more fuel. It demands considerable experience and familiarity with the method. The high-boiling and somewhat water-soluble oil constituents cannot be completely vaporized or they require large quantities of steam. Thus, the process becomes uneconomical.

2.3.2 CO2 and Super Critical CO2 Extraction

The most modern technologies, Carbon Dioxide and Supercritical Carbon Dioxide extraction involve the use of carbon dioxide as the 'solvent' which carries the essential oil away from the raw plant material. The lower pressure CO_2 extraction involves chilling carbon dioxide to between 35 and 55 degrees F, and pumping it through the plant material at about 1000 psi. The carbon dioxide in this condition is condensed to a liquid. Supercritical CO_2 extraction (SCO_2) involves carbon dioxide heated to 87 degrees F and pumped through the plant material at around 8,000 psi – under these conditions; the carbon dioxide is likened to a 'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the essential oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance (Pandey, 2006).

2.3.3 Soxhlet extraction

A Soxhlet extractor is a piece of laboratory apparatus (Harwood, 1989) invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analyte into the bulk liquid (Schantz, 1998). This procedure is repeated until virtually complete extraction is achieved. There are several advantages of Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed.

2.3.4 Cold Pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is fully pure and retains all the properties of the plant. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, (Arnould-Taylor, 1981). In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

2.4 Factors affecting the oil yield

The following factors are believed to have considerable effect on the oil yield. Each factor is seen in detail from the different literatures cited.

2.4.1 Effect of drying methods on the oil yield

Dry herbs have a great importance, not only for the food purposes, but also for medicinal uses (Hedrick, 1972). 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. The drying of mint is an effective method that increases the shelf life of the final product. However, drying causes changes in the product mainly associated with fragrance and appearance (Diaz-Maroto, 2003). Drying of the plant material can be achieved by several processes.

Mint is regarded as one of the most important spices throughout the world. Mint leaves in botany are the common name for members of the Labiate, a large family of chiefly annual or perennial herbs. The essential oils of mints are widely used as flavorings in the food, spicing, tea infusions, cosmetic and pharmaceutical industries (Özbek, 2007). The study made by Tamás Antal, Benedek Kerekes, László Sikolya ,2010 has examined the influence of two different drying methods (freeze-drying and hot-air drying) on the volatile compounds of spearmint. The effect of the pressure (high and low) of the freeze drying procedure was also investigated. And they concluded that the quality of dried spearmint depends on the drying method. From the value of the nine components, it can be concluded that best aroma value was achieved in products dried by *FD-HP*, followed by *FD-LP* and *HAD*. *FD-LP* shortened the drying time, revealed the lower volatile content. Increasing the pressure in the drying chamber considerably raised the release of volatile compounds. Goodness of fit of the experimental data by four drying models was determined by comparing coefficient of determination. The third-degree polynomial model showed a good fit curves than other models. However, sigmoid model provided some practical information regarding the time coordinate *c* of the critical point, which divides the drying kinetics into the two drying periods (Tamás Antal, 2010).

Drying reduces moisture content of harvested biomass decreasing the biomass three to four times relative to the fresh herbage at harvest. Hence both transportation and energy costs associated with the distillation are reduced

Volatile aroma compounds are the most sensitive components in the process of drying. The effect of drying on the component of essential oil of various aromatic plants, fruits and vegetables has been the subject of numerous studies, which show that the changes in the concentration of the volatile compounds during drying depends on several factors, such as drying method and drying conditions (Kripanand, 2015)

Here in this research the effects of different drying methods on the oil yield are investigated but the effect of these drying methods on the chemical composition were not covered. And the different drying methods considered in this study are the following three.

1. **Shade Drying**

From different researches done before it was found that the highest essential oil contents was observed in plant material dried under shade followed by oven drying methods. The drying methods did not affect the composition of the essential oil. However, shade- drying method could be suggested as the more convenient method to isolate mint essential oil (Tuncay ÇALIŞKAN1, 2017).

2. Sun drying

Sun drying affects to some of the oil constituents according to the study conducted by Valtcho D. Zheljazkov .Drying of the spearmint biomass under direct sun increased the concentration of beta-pinene but reduced the concentrations of myrcene and germacrene in the oil relative to drying under shade (Zheljazkov, 2014).

3.Oven drying

The oven drying is usually done at a temperature range of40 – 50oc and the effects on the yield are recorded. Oven-drying can cause changes in oil properties and degradation in terms of color reaction and decomposition of active ingredients (Abu-Goukh, 2015)

2.4.2 Distillation time effect on the yield of essential oil

A study titled "Effect of hydro distillation process on oil recovery of fresh Mentha leaves "was conducted by Karanvir Gill and Rahul Gupta and said that most of the oil was extracted in the first 20 minutes. It might be mainly due to the fact that diffusion of oil was fast due to high initial oil content. This diffusion rate decreased significantly when the oil content of leaves decreased (Gill, 2014).

2.4.3 Storage of the Plant Material

The storage of plant material before size reduction also causes some damage in the way of ultimate loss of volatile oil. It should be stored in its natural condition. Gradual evaporation results in some loss under these circumstances, the major sources of loss being represented by oxidation and resinification of the essential oils. If the plant material must be stored before processing; it should be kept in a dry atmosphere at a low temperature and in a room free from air circulation if possible in an air-conditioned storehouse. All such losses are obviously avoided if the plants are processed immediately

The volatile oil enclosed in the plant tissue is usually in one way or another affected by the drying of the plant material after the harvest.. Some fresh plants, or parts, with a high water content lose much of their essential oil by air drying; others very little.

This loss is caused by evaporation, oxidation, resinification and other chemical actions. Contrary to expectation, evaporation here seems to play a subordinate role to oxidation and resinification. Indeed, actual evaporation of the volatile oil through the walls of the plant tissue cannot take place readily because the oil must first be brought to the surface through hydro diffusion, with water or plant moisture acting as a carrying medium. Thin walled flowers and leaves present no obstacle to the forces of diffusion, and in most cases evaporation will affect the more water-soluble constituents of a volatile oil rather than the low boiling terpenes (GUENTHER, 1948).

As per the study conducted by Salim storage of the produced oil has effect on the constituents, Carvone content progressively decreased with an increase of storage period of spearmint herb. Other spearmint herb constituents act differently, some of which decreased in amount and other appeared or disappeared. This change in chemical constitutes are due to oxidative changes in the presence of air; oxygen; temperature and light during storage at semi-arid ambient temperature (Salim, 2016).

2.4.4 Overall production process of the spearmint essential oil

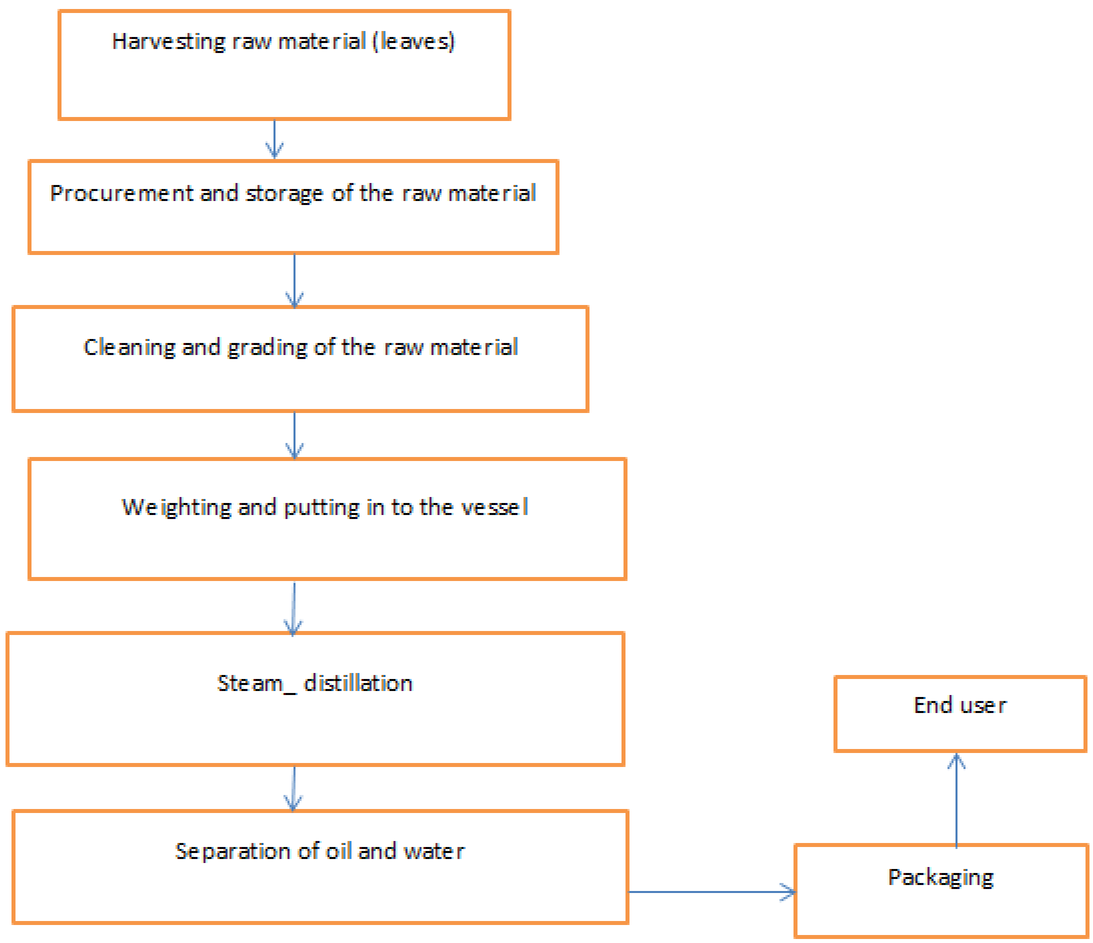


Figure 2.3 flow chart of spearmint oil production

2.5 Physical and Chemical Properties of Spearmint Oil and Carvone

In the essential oil industry, the quality of the mint oil is evaluated based upon

Various characteristics, such as composition of the individual terpene constituents, specific gravity refractive index, etc. (Fenaroli, 1975).

Table 2.1 properties of spearmint oil

Property	**Characteristic/Value**	**Source**
Percent Composition	Varies: For spearmint oil includes DL-carvone (46-68%), limonene (6-20%), pinenes (1-5%), linalool, cineole, and various terpenoids, alcohols, esters, ketones, aldehydes, acids, phenols and other compounds.	(Lawrence 2007)
Physical state at 25°C/1 Atm	liquid	(Merck 2015)
Color	Colorless, yellow or greenish-yellow	(Merck 2015)
Odor	Characteristic spearmint odor and taste	(Merck 2015)
Density/Specific Gravity	0.917-0.934	(Merck 2015)
Melting point	Not found	
boiling point	230 ^{o}c	(Royal Society of Chemistry 2015)
Solubility	Slightly soluble in water; 1:1 in 80% ethanol at 20°C	(Merck 2015)
Vapor pressure	Carvone: 0.16mm Hg	Not found

pH	N/A	
Viscosity	Not found	
Flammability	Flash point 94°C (closed cup); Slight fire potential	(Sigma-Aldrich, Inc. 2014;)
Storage stability	Volatile—store in an airtight container	(Sigma-Aldrich, Inc. 2014)

2.5.2 Chemical Properties

1. Acid value

Most essential oils contain only small amounts of free acids. Consequently the acid content is usually reported as an acid number rather than as a percentage calculated as a specific acid.

The acid number of oil is defined as the number of milligrams of potassium hydroxide required to neutralize the free acids in 1 g. of oil. The acid number of and oil often increases as the oil ages, especially if the oil is improperly stored; processes such as oxidation of aldehydes and hydrolysis of esters increase the acid number. Oils which have been thoroughly dried and which are protected from air and light show little change in the amount of free acids.

2.Peroxide value

Peroxide value is one of the most widely used test for oxidative rancidity in oils. It is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation. Generally, the peroxide value should be less than 10 mg/g oil in the fresh oils

3. Saponification Value

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat.

The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid.

4. Iodine Value

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of the oil/fat, when determined by using Wijs solution. The iodine value is a measure of the amount of unsaturation (number of double bonds) in oils.

2.6 Spearmint oil Chemical reactions

Chemical changes in volatile compounds are known to occur due to environmental conditions, such as light, oxygen, moisture, heat and storage period resulting in the formation of artifacts or polymerization. Essential oils are exposed to oxidative changes in the presence of air and light, and during storage. These changes usually have severe effects on flavor quality; most flavoring materials are to some extent heat sensitive. The degree of change is usually a function of both temperature and time and less stable compounds may also change due to chemical interactions with other constituents .Changes in essential oil components during storage could occur by rearrangement, hydrogenation or dehydrogenation of other components (Salim, 2016) To obtain new aromatic products of a better quality or value, with pleasanter sensations, we Can use:

-Esterification (mint).

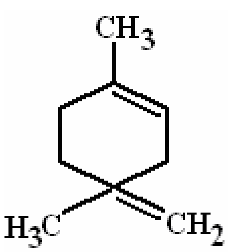
-Hydrogenation.

-Hydration

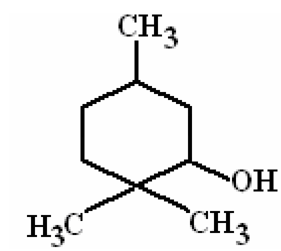
Major components in spearmint oil are carvone, limonene, and 1, 8-cineole. Essential oil compositions of aromatic plants depend on their genetic structure, the climatic factors and the agronomical practices as well as harvest and post- harvest managements. In addition, harvest time (cut) and phonological stages can affect herbage yield and oil content and composition of the plants (Sourestani, 2016)

Carvone is one of many monoterpenes present in spearmint oil. Monoterpene biosynthesis begins with geranyl diphosphate. Cyclase enzymes isomerize geranyl diphosphate to linalyl pyrophosphate, which is then cyclized to form (-)-limonene. In spearmint plants, the enzyme (-)-limonene-6-hydroxylase hydroxylates limonene at the C6 position to produce (-)-trans-carveol, which is then oxidized to form (-)-carvone. From there, (-)-carvone can undergo various oxidation and reduction reactions to form isomers of dihydrocarvone, dihydrocarveol, carvyl acetate, and dihydrocarvyl acetate. Other monoterpenes in spearmint are formed from the hydroxylation of (-)-limonene by (-)-limonene-6-hydroxylase or synthases that work on linalyl diphosphate (Lawrence, 2007)

Some common monoterpenes and their oxygen derivatives are:



(Limonene)



(Menthol)

The industrial preparation of carvone (according to *Ullman's Encyclopedia of Industrial Chemistry*) is as below.

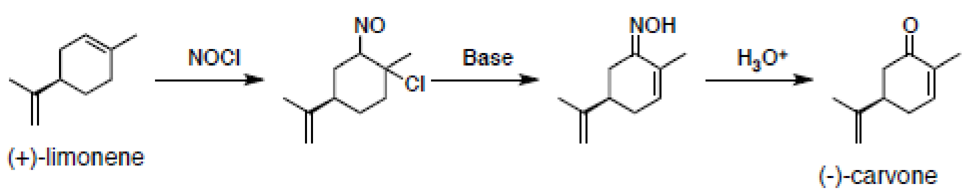


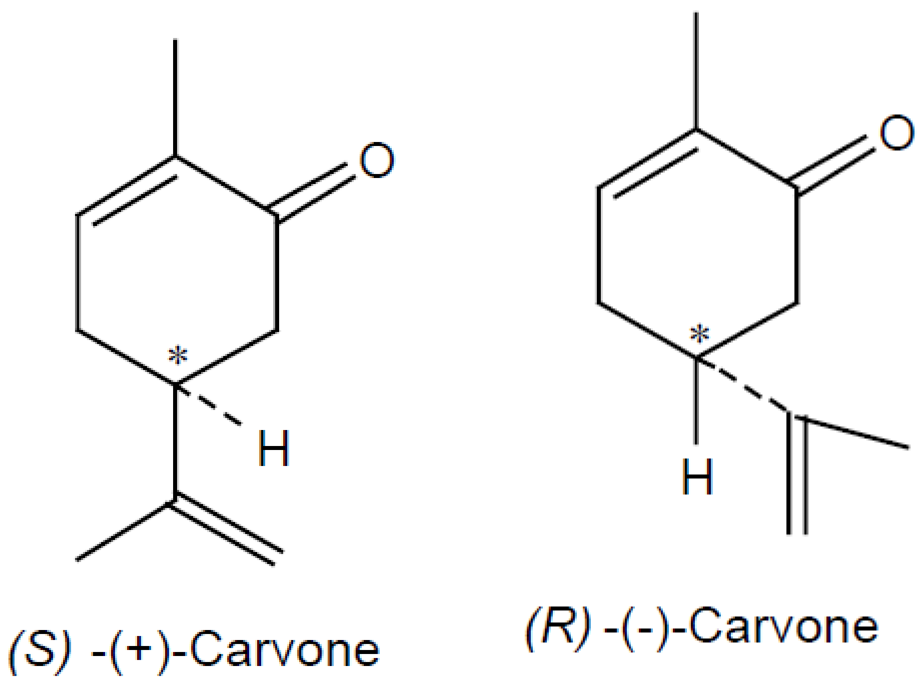
Figure 2.4 carvone preparations from limonene

2.6.1 L- Carvone

Carvone being the main constituent in spearmint oil is taken as a measure of quality of the oil. Carvone can also be found from other sources like that of caraway seeds. This carvone from caraway is similar with that of spearmint. All the physical properties are identical except for the optical rotations of the two isomers (enantiomers), which are of opposite sign. Thus, for both (+)

- and (-) - carvone, the infrared, nuclear magnetic resonance spectra, the gas chromatographic retention times, the refractive indexes, and the boiling points are identical. Hence, the only difference in properties one can observe for the two carvone isomers are the odors and the signs of rotation in a Polari meter.

L-Carvone imparts the odor of spearmint and constitutes 50-65% of the essential oil from the flowering tops of the spearmint plant Mentha spicata L. (M. viridis L.) Alternatively, l-carvone can also be synthetized from d-limonene ((EFSA), 2014)



From caraway seed from spearmint

Fig 2.5 the two isomers (enantiomers) of carvone, which are of opposite, sign

2.6.2 Spearmint oil properties by country

The different types of the spearmint oil in different countries are the following

American spearmint Oil.

Specific gravity	0.92-0.94
Refractive index	1.480 to 1.489
Acid value	0 to 2
Ester	18 to 36
Carvone	35-66 %

English Spearmint Oil

Specific gravity	0.926 to 0.935
Optical rotation	- 39° to - 52°
Carvone	30 to 48 %

Austrian Spearmint Oil.

Specific gravity	0.936 to 0.952
Refractive index	1.4890 to 1.4930
Carvone	61 to 72 %

Russian Oil.

Specific gravity	0.880 to 0.890
Carvone	5 to 10 %
Linalol	50 to 60%

2.6.3 Gas Chromatography-Mass Spectrometry

Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios (*m/z*) and relative abundances (Joulain, 1998). The analyte may be ionized thermally, by an electric field or by impacting energetic electrons, ions, or photons.

During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis.

In addition, the potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of data generated by GC-MS instruments was fully exploited. The most frequent and simple identified cation method in GC-MS consists of the comparison of the acquired unknown mass spectra with those contained in a reference MS library.

A mass spectrometer produces an enormous amount of data, especially in combination with Chromatographic sample inlets. Over the years, many approaches for analysis of GC-MS data have been proposed using various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantify all of the chromatographic peaks. Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds.

However, as is well known, compounds such as isomers, when analyzed by means of GC-MS, can be incorrectly identified; a drawback which is often observed in essential oil analysis. As is widely acknowledged, the composition of essential oils is mainly represented by terpenes, which generate very similar mass spectra; hence, a favorable match factor is not sufficient for identification and peak assignment becomes a difficult, if not impracticable, task. In order to increase the reliability of the analytical results and to address the qualitative determination of compositions of complex samples by GC-MS, retention indices can be an effective tool. The use of retention indices in conjunction with the structural information provided by GC-MS is widely accepted, and routinely used to confirm the identity of compounds. Besides, retention indices when incorporated to MS libraries can be applied as a filter, thus shortening the search routine for matching results, and enhancing the credibility of MS identification.

According to D. Joulain and W. A. Konig, provided data contained in mass spectral libraries have been recorded using authentic samples, it can be observed that the mass spectrum of a given sesquiterpenes is usually sufficient to ensure its identification when associated with its retention index obtained on methyl silicone stationary phases. Indeed, for the cited class of compounds, there would be no need to use a polyethylene glycol phase, which could even lead to misinterpretations caused by possible changes in the retention behavior of sesquiterpenes hydrocarbons as a result of column aging or deterioration. Moreover, according to the author attention should be paid to the retention index and the mass spectrum registration of each individual sesquiterpenes, since many compounds with rather similar mass spectra elute in a narrow range; more than 160 compounds can elute within 100 retention index units on a methyl silicone-based column.

2.7 Application of spearmint oil

Spearmint, commonly known as minty is used as breath freshening flavoring agent, oral hygiene products including gums and candies. Its different applications are detailed below.

I. cosmetics

Perfume is a substance that releases and sprays a fragrant odor. It is a very volatile liquid distilled from a plant part. The essential ‘plant distillates’ (essential oils) interrelate with the human body by four distinct modes of action – pharmacological, physiological, psychological and spiritual. Our body uses the aromatic molecules (essential oils) in two ways: (1) through our olfactory system which is connected to the brain where our most primal feelings, urges and emotions reside, and (2) by absorption of the low molecular weight compounds of essential oils through skin (Vankar, 2004).

While being used in cosmetic products the allergenic level should be given a focus. Some of the spearmint concentrations and applications in the USA fragrance are given as below

Table 2.2 spearmint concentration limits

Tyepe of cosmetic product	Concentration of spearmint in%	
	usual	maximum
Soap	0.02	0.15
Detergent	0.002	0.015
Creams, lotions	0.01	0.05
Perfume	0.08	0.4

Acute toxicity: The acute oral toxicity was reported as approximately 5 g/kg,

The acute dermal is > 2 g/kg and (Moreno, 1976)

Irritation > 4%

The other application of natural essential oils is the so called alternative or natural medicine. Although relatively new it is expanding very fast and its discoveries are also used in cosmetic and pharmaceutical industries where good odor and healing properties are combined in the products (SILVA, 1995).

II. Medicinal therapy

These medicinal applications include carminative, anti-inflammatory, antispasmodic, antiemetic, diaphoretic, analgesic and stimulant application. It can be further used in the treatment of nausea, anorexia, ulcer, bronchitis, sinus toothache, itching and skin irritation, cold and flu, headache muscle pain, infections caused by bacteria and virus. Release stress, mental exhaustion and depression, and it helps in strengthening the immune system. It also acts as mosquito repellent (Gokalp Iscan, 2002). Mint oil showed excellent antimicrobial properties; hence it acts against the bacteria that affect food and food-borne pathogens.

Mint oil as antioxidant

Mint oil from the genus *Mentha* is comprised of many components, some which because of their interconversion many oxidation-reduction reactions may take place, as said by Craig Landsburg, 1999, exhibit antioxidant activity. (+)-Pulegone is the most oxidized of the major mint oil constituents and (-f methyl acetate is the least oxidized (the most reduced). It is speculated that due to the oxidation level (-)-Mentha acetate may serve well as an antioxidant followed in antioxidant capability by (-)-menthol, (-)-Mentone and (+)-Pulegone can theoretically be expected to have the poorest antioxidant capability because of its highly oxidized chemical structure.

Generally, it is believed that the antibacterial activities of essential oils are likely related to the percentage of terpenes, and aldoketones. 1,8-Cineole , limonene and *cis*-carvone oxide have been already reported to possess antibacterial activities which may explain the good antibacterial activity of the present essential oil which is rich with these three components (Kabouche, 2016)

The peroxide test determines the extent of primary oxidation reaction which has occurred by measuring the different concentrations of hydroxides and peroxides. Peroxide Value (PV) is obtained via the reaction of thio sulphate and peroxide using starch and iodine to indicate the end point Peroxide values are expressed in units of mill equivalents of Peroxides per kg of sample

2.7.1 Further Uses of Spearmint Oil

The uses of spearmint oil extend beyond the kitchen and the medicine cabinet. For instance, it can be used to help the mind relax or to instill positive emotions. The rejuvenating fragrance is said to help clean the body emotionally and mentally. A list of spearmint oil’s potential uses is as below:

- Aromatherapy oil — Because of its menthol content, spearmint oil is often used in aromatherapy to help ease fatigue, headaches, migraines, nervousness and even digestive problems.
- Food ingredient- the oil of spearmint is sometimes added to baked goods, frozen dairy, meats, beverages and candy. Note, however, that you are better off consuming whole, raw foods than these processed ones.
- Fragrance -This essential oil is added to certain types of perfume. It is commonly mixed with other herbal oils like jasmine and lavender.
- Ingredient in dental care products — It is often added to gargles and toothpastes
- Pest and insect repellent --this oil can ward off insects like mosquitoes, as well as mice and other rodents.

Spearmint essential oil can be used in a number of ways:

- Inhalation Compared to peppermint oil, spearmint oil is milder and can be used around children. Inhaling its fragrance may help fatigue and mental stress.
- Topical application — Add a few drops of this oil to your body care products and it may help ease acne and other skin conditions. Rubbing a diluted solution over your stomach may also ease digestive issues.
- Taken internally ingesting spearmint oil can help treat digestive problems. However, this should never be done without the aid of a professional aroma therapist or your physician.
- Added to bath water this may help relieve fatigue, fever and muscle pain.

2.7.2 Safety

Referring to European food Safety Authority (EFSA) the following dosages of spearmint oil in different applications are mentioned.

Table 2.2 spearmint concentration limits

application	Average usual (ppm)	Average Maximum(ppm)
baked goods	1055	1320
beverages(nonalcoholic)	110	140
beverages(alcoholic)	120	155
chewing gum	8000	24000
condiments / relishes	50	250
frozen dairy	50	130
gelatins / puddings	90	95
hard candy	5000	10000
jams / jellies	72	1900

3. MATERIALS AND METHOD

3.1 Materials

The experimentation was done in School of Chemical and Bio Engineering laboratory at Addis Ababa Institute of Technology.

The different materials and chemicals involved in the production and characterization of the spearmint oil are tabulated below. The following list of main equipments, were used for the research.

Table 3.1 equipment list

no	name	used for
1	Distillation apparatus	Extraction of the spearmint oil
2	GC-MS device	Analyzing the constituents
3	Measuring cylinders and beakers	Measuring volume and sample handling
4	Funnel filter	Separation of the oil and water
5	Burette and pipette	Titration in SV determination
6	Electronic mass balance	Sample weighting

Table 3.2 list of chemicals

no	Chemical name	used for
1	Potassium hydroxide	Saponification value
2	Hydrochloric acid	Titration of sample
3	Ethanol	AV(acid value)
4	Chloroform	PV(peroxide value)
5	Acetic acid	PV
6	Potassium iodide	PV
7	Sodium thio sulphate	PV
8	Carbon tetra chloride	IV(iodine value)

3.1.1 Sample preparation

The sample spearmint leaves were collected from nearby home gardens in Addis Ababa around Gerji area and some were bought from the vegetables market. The leaves and the tops of the spearmint plant were cut to separate from the stem and roots and dried at different drying conditions and for different drying durations. The three drying conditions or methods were shade drying, sun drying and oven drying. And the three levels of drying duration were 0 days just after some 2-4 hours to distillation, 3 days and 7days of drying of by the methods mentioned and the oven drying duration was 24 hours drying at a temperature of 46 ^{o}c.

3.1.2 Raw material analysis

The moisture content was determined by heating the sample in an oven for 24 hours. Three trials were done to measure the moisture content of fresh spearmint leaves and the average of these three records is reported to be the moisture content of the spearmint leaves.

Size reduction was performed to about 5-10 mm by hand. This size was taken for all experiments considering that the small the size the greater the yield and just to investigate the below mentioned factors the smaller size possible was taken as constant.

Furthermore the ash content and organic matter content were determined. The moisture content of fresh leaves was 71%, ash content 4.3% and organic mater 24.65%

3.2 Methods

Hydro-distillation was chosen for the extraction of the oil. And the oil produced was optimized on the mass % basis.

3.2.1 Experimental set up

The extraction process (hydro distillation of the herb) was conducted at the AAiT lab. The experiment was begun after the required setup and process variables were adjusted with three trials. The test runs were conducted according to the design stated below in 3.2.3. The following modefica of distillation apparatus was used

After the raw material is immersed to the water in the seven liter pressure cooker which was filled up to half level. Heating it on a stove the vapor containing the oil required is then made pass through a cooling coil having three turns. And the distillate is collected on the plastic bottle connected to the end of the cooling coil which penetrated the container holding the cooling water.

Fig 3.1 experimental set up

3.2 Experimental design

For the lab experimental works the general factorial design was chosen to test the effects of three factors on the yield of oil produced. Twenty-seven number of experiments were conducted to know how affecting the factors are to the optimum yield of the oil.

Table 3.3 factors and their levels

factors	**levels**		
Drying method	Shaded drying	Sunlight drying	Oven drying
Drying duration	0 days	3 days	7 days
Distillation retention time	1 hour	1.5 hours	2 hours

3.4 Characterization and analysis

The sample product oil (the mixture of maximum A1B3C3= 2.27 and second maximum A3B3C3=2.22 %) was analyzed by GC-MS at Jije laboratory (Mass Hunter GC/MS Acquisition B.07.03.2129 18-May-2015 Copyright © 1989-2014 Agilent Technologies, Inc.) for its constituents and the different characterization titration experiments were exercised to determine the different chemical properties. And the physical properties were measured by their respected meters at SCBE lab.

3.4.1 Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the substance.

$$\boldsymbol{SV = 28.05(b - a)/w} \quad \textbf{(3.1)}$$

a : Titration volume (ml)

b : Blank level (ml)

28.05=constant

w: Sample size (g)

3.4.2 Acid value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance

2 g of the sample was weighed into a 250-mL flask and 50 ml of a mixture of (25 ml ethanol and 25 ml of ether) was added and adding 1 ml of phenolphthalein it was titrated with potassium hydroxide (0.1 M), constantly shaking the contents of the flask until a pink color was obtained. The number of mL required was noted (a).

The acid value was calculated from the following formula:

Av= (a*5.61)/weight of sample (g)……………………….. (3.2)

3.4.3 Peroxide value

2 gram (V_2) of the sample oil was dissolved in conical flask by adding 10 ml of chloroform and 15 ml of glacial acetic acid. 1 ml of a freshly prepared solution of potassium iodide was added, mixed by gentle swirling and set aside in the dark for 5 minutes. Then it was titrated with sodium thio sulphate (0.1 M) using starch as indicator. Repeating the operation without the substance being tested (V_1) and the difference between the titrations was calculated as:

PV= ((V2_ V_1)*100)/weight of sample (g))………………….. (3.3)

3.4.4 Iodine value

The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance.

Based on Hanus method

A 1.68 g of the test sample (spearmint oil) was accurately weighed in a dry 250-ml flask; 15 ml of carbon tetrachloride was added and dissolve. Then is added 25 mL of iodine bromide, the stopper previously moistened with potassium iodide was then inserted after that the flask was shaken gently and kept in the dark for 30 minutes. Some mL of potassium iodide and 150 ml of water are added shaking the contents of the flask and titrated with sodium thiosulfate (0.1M). Starch was added as indicator towards the end of the titration. The number of ml required (a) was recorded. At the same time in exactly the same manner the operation was carried out but without the substance being tested, and the number of mL of sodium thiosulfate (0.1M) required (b) was noted. The iodine value was calculated from the following formula:

$$\boldsymbol{IV = (b - a) * \frac{1.269}{weight\ of\ sample(g)}} \qquad (3.4)$$

4. RESULT AND DISCUSSION

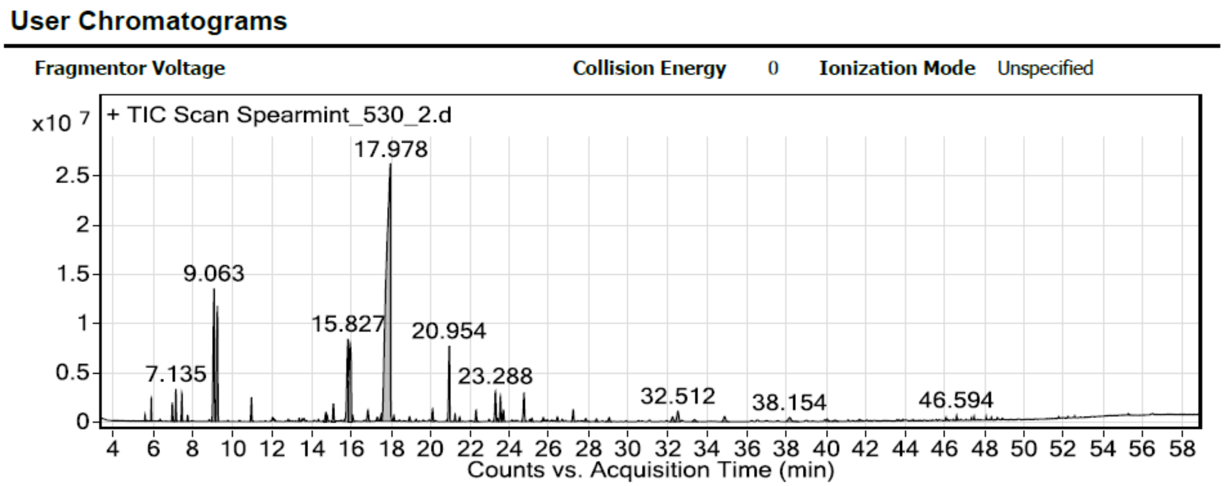
4.0 GC-MS analysis

From the results of the GCMS total of 41 compounds were found in the spearmint oil and the dominant constituents were found to be

1. (-)-carvone (50.65%)
2. D-Limonene (7.85%)
3. 1,6-Dihdro carveol (7.23%)
4. Trans-Dihydrocarvone (6.19%)
5. (-)-Dihydrocarvyl acetate (3.85%)
6. Caryphllene (1.63%)
7. (-)-beta-bourbonene (1.58%)

The carvone content of spearmint oil according to the different studies made before is in the range of 40-70 % .And the carvone share of this research is found to be 50.65%, which is in the range mentioned above. This high carvone content gives the oil a characteristic spearmint odor which makes it effective to be used as perfumery additive in soap making and different cosmetic products.

Referring to the peaks of the chromatogram the highest peak was that of **carvone** (17.978) and the shortest time was that of **5, 5-Dimethyl-1-vinylbicyclo [2.11] hexane** (5.58 minutes). And these figures indicate that the higher the peak the larger the area and this tells us the quantitative abundance of carvone. And the shorter the retention time the more volatile the component is **5, 5-Dimethyl-1-vinylbicyclo [2.11] hexane**.



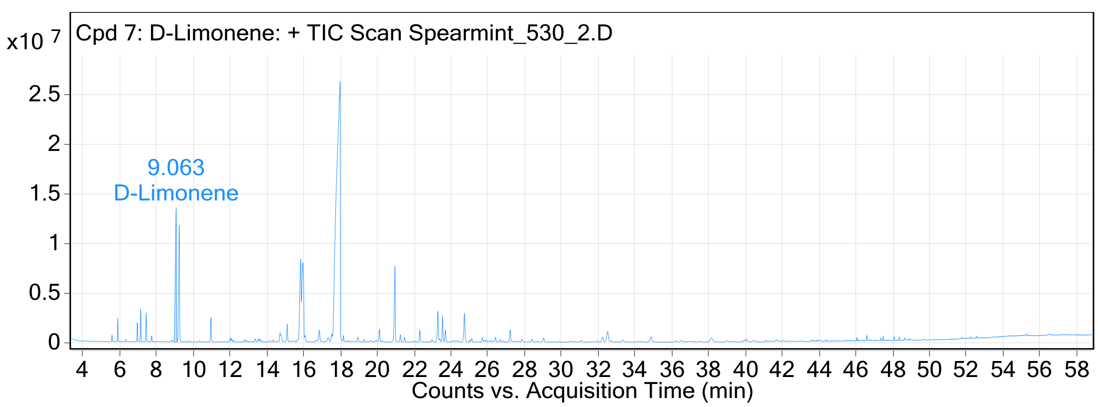


Fig 4.1 chromatograms

4.1. Yield of Oil Extracted from spearmint

Table 4.2 oil yield of spearmint at different levels of the three factors

Runs	**Factor A**	**Factor B**	**Factor C**	**Oil yield (%)**
23	Shade drying	0 days	1 hour	**1.87**
18	sunlight	0 days	1 hour	**1.87**
8	Oven drying	0 days	1 hour	**2.05**
26	Shade drying	3 days	1 hour	**1.89**
11	sunlight	3 days	1 hour	**2.02**
2	Oven drying	3 days	1 hour	**1.91**
13	Shade drying	7 days	1 hour	**1.89**
17	sunlight	7 days	1 hour	**1.9**
9	Oven drying	7 days	1 hour	**1.92**
1	Shade drying	0 days	1.5 hours	**2**
14	sunlight	0 days	1.5 hours	**1.98**
6	Oven drying	0 days	1.5 hours	**1.99**

24	Shade drying	3 days	1.5 hours	**2.1**
15	sunlight	3 days	1.5 hours	**2.03**
10	Oven drying	3 days	1.5 hours	**2.01**
12	Shade drying	7 days	1.5 hours	**2.04**
3	sunlight	7 days	1.5 hours	**2.08**
25	Oven drying	7 days	1.5 hours	**2.12**
4	Shade drying	0 days	2 hours	**2.15**
27	sunlight	0 days	2 hour	**2**
19	Oven drying	0 days	2 hours	**2.01**
20	Shade drying	Shade drying	2 hours	**2.05**
5	Sunlight	3 days	2 hours	**2.1**
16	Oven drying	3 days	2 hours	**2.11**
22	Shade drying	7 days	2 hours	**2.27**
21	Sunlight	7 days	2 hours	**2.16**
17	Oven drying	7 days	2 hours	**2.22**

4.2. Data Analysis

The statistical analysis for the spearmint oil extraction was done by analysis variance (ANOVA). As shown in table 4.2 from the ANOVA results of oil extraction, the multiple regression analysis of the experimental data suggested a linear equation, which was modified to discard the insignificant AC and AB model terms. The reduced linear model developed shows the interaction between the dependent oil yields (Y) and the coded values of the independent variables A, B, and c which represent the factors drying method, drying duration and distillation time respectively.

The significance and adequacy of the model were tested using ANOVA. It was observed that from table C2 in appendix C the linear interaction effects are due to the Factors drying duration (B) and distillation time. The effects of drying duration (B) and distillation time are significant based on their p>F values. Their p values are less than 0.05 while that of A(drying method) is found to be 0.3344 which is > 0.05 and hence insignificant.

From the ANOVA data obtained it can be observed that the interaction effects of the drying method and drying duration as well as that of drying method and distillation time ,AB and AC are not significant whereas BC is significant as its p>f value is 0.0196 which is less than 0.05

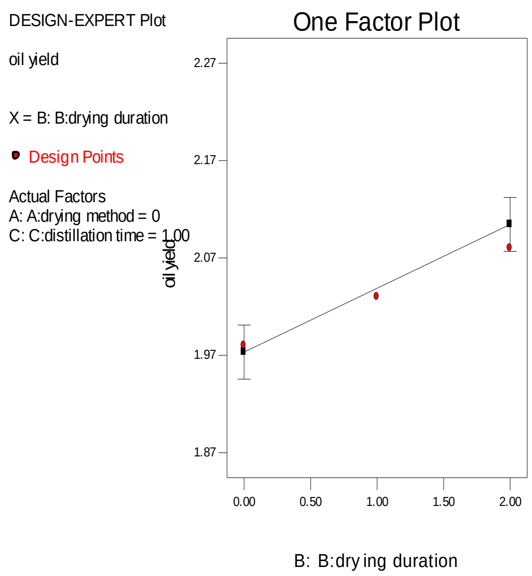
4.2.1 Regression model equation

Oil yield = 2.03+0.011*A[1]-0.018*A[2]+0.066*B+0.1*C

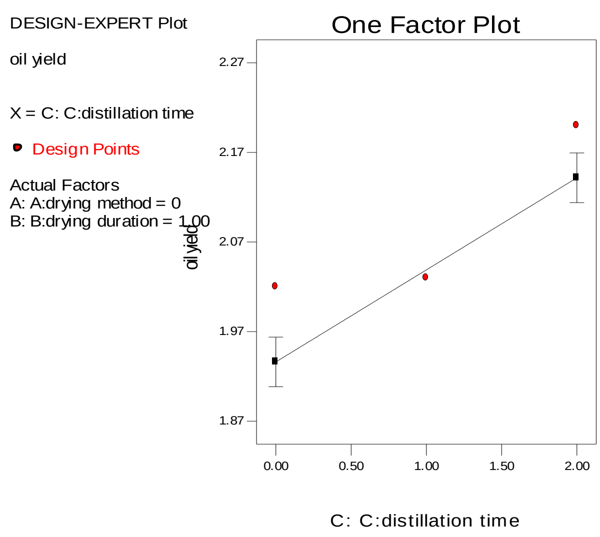
As factor A is not significant, terms containing it are neglected

Yield(Y) =2.03+0.066*B+0.1*C

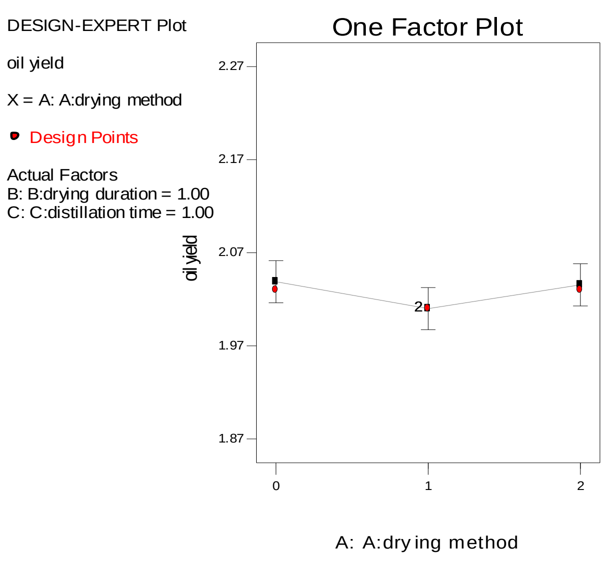
4.3 Single and Interaction Effect of the three Factors



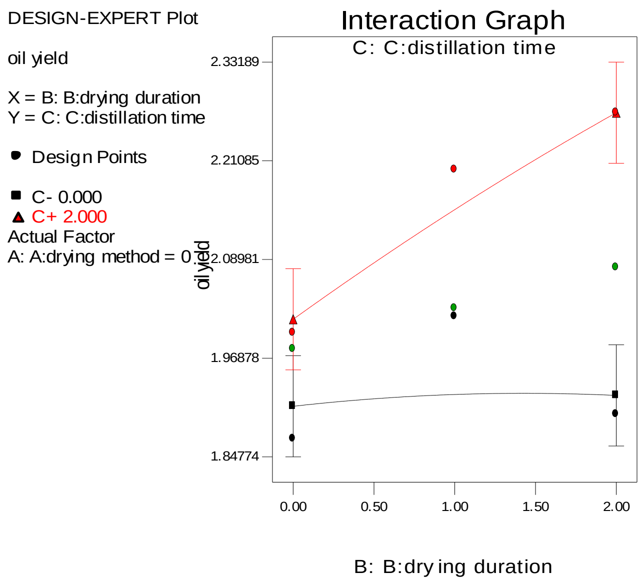
a)effect of drying duration



b)effect of distillation time



c) Effect of drying method



d) Interaction effect

Fig 4.2 interaction effect of the three factors

Table 4.3 ANOVA

Std.dev.	0.043
Mean	2.03
CV	2.14
PRESS	0.1
R-Squared	0.9233
Adj R-Squared	0.8465
Pred R-Squared	0.6713
Adeq Precision	12.903
Std.dev.	0.043
Mean	2.03
CV	2.14
PRESS	0.1
R-Squared	0.9233
Adj R-Squared	0.8465
Pred R-Squared	0.6713
Adeq Precision	12.903

From the table 4.3 above it can be seen that the Adjustable R-squared and the predicted R squared are in good agreement 0.8465 and 0.6713respectively. Furthermore the ratio being 12.903 >makes the result to be significant.

4.4 Characterization of spearmint oil

The different chemical properties of spearmint oil are performed according to the procedures and formulas in chapter three and here are the results that are obtained from the lab activities.

Table 4.4 physicochemical properties of spearmint oil

Property of oil	Value measured	Commercial value(EFSA)
Density	0.915	0.91700 to 0.93400 @ 25.00 °C.
Refractive index	1.43	1.47900 to 1.48900 @ 20.00 °C.
Viscosity	0.47mPas	N/A
pH	6.06	N/A
Saponification value (mg KOH/g)	41.83	N/A
Acid value(mg KOH/g)	0.045	0.56
Peroxide value (meq /kg)	0.67	N/A
Iodine value (g iodine/100g oil)	17.8	**N/A**

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research oil was extracted from spearmint leaves by hydro-distillation method. And from the results discussed in chapter 4 it can be concluded that the oil analyzed has fair yield. Drying method, drying duration and distillation time were the three factors considered for the optimization of the oil yield. Under this investigation drying method (shade, sun and oven drying), drying duration (0, 3, 7 days) and distillation time (1, 1.5 and 2 hrs.) were considered. For this hydro-distillation, the maximum oil yield was found to be 2.27% on weight basis. This was obtained at distillation time of 2 hours, shade dried for a duration of 7 days. And the minimum oil yield of 1.8% was found at the distillation time of 1 hour, sun dried for 0 days (3 hours after harvest. Therefore, increasing drying duration and distillation time increases the oil yield.

The effect of the coded parameters B and C was found to be significant ($p<0.001$) and drying method affects less to the oil as can be referred from the graph of effect of the single factor A (drying method) which is almost horizontal and more over the p value from the ANOVA is 0.3344 which is > 0.05 and hence indicates the insignificance of the mentioned factor.

The GC/MS analysis of the spearmint oil showed the dominant component is carvone (50 %). This component gives the oil its characteristic spearmint odor. Next to carvone the dominant components are D-Limonene (7.85%), 1, 6-Dihdrocarveol (7.23%) Trans-Dihydrocarvone (6.19%) and (-)-Dihydrocarvylacetate (3.85%).From all the 41 compounds found eugenol, the known allergenic substance was not found in the oil and this is an advantage of the oil to be used in different cosmetics and soap products.

Regarding the physicochemical properties the Av (0.045), which is well below 10, PV (0.67), SV (41.83), IV (17.8) it was found to be color less having a density of 0.915, viscosity of 0.47 mPa.s at 26 .7 ^{o}c and refractive index of 1.43.

5.2 Recommendations

Spearmint plants are strong aromatic that need to be utilized for different cosmetic products and food flavors. In spite of its importance and impressing fragrance it is not seen to be planted widely in Ethiopia except in some small home gardens and research centers (Wendo genet research area).

This research has shown the possibility to extract a fair amount of essential oil from spearmint leaves using hydro distillation method. The factors considered were investigated for their effect on the oil yield and the oil constituents are analyzed by GC-MS, mainly to see the dominant compounds like carvone and limonene. The researcher recommends:

- Further studies to be done on spearmint oil considering more factors
- Studies could look for the effect of pressure and temperature, using pressure and temperature controlled extraction methods.
- The effect of different drying temperatures on the oil constituents that is oil product of every run could be subjected to GC/MS test.

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APPENDIXES

APPENDIX A: photos of some activities

Acid value determination   iodine value titration


JENWAY
6.06
3505 pH Meter


SV-10
0.47 mPas
26.7 C

APPENDIX B: GC/MS result from Jije lab

JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Page 1 of 3	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			

Customer Name	Gebremichael G/hiwot	Test Report No	121
Contact Person:	Gebremichael G/hiwot	Reported Date:	18/04/2019
Sample Type:	Spearmint Leaves Essential Oil	Test Request No:	Not Specified
Sample Source:	Not Specified	Tel:	+251 920 57 17 87
Sample collected by:	Customer	Fax:	Not Specified
Sample Collected Date:	10/04/2019	E-mail:	gimwot@yahoo.com
Sample Received Date:	15/04/2019	Tested By:	LE-02
Sample Condition	Normal	Date Tested:	16-17/04/2019

S/N	Name	Formula	RT	Area	Area %
1	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	C10H16	5.58	1139121.00	0.15
2	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	C10H16	5.89	4512211.00	0.61
3	Sabinene	C10H16	6.96	4457886.00	0.60
4	Beta-Pinene	C10H16	7.14	8001268.00	1.08
5	Beta-Myrcene	C10H16	7.43	7141366.00	0.96
6	3-Octanol	C8H18O	7.73	1547001.00	0.21
7	D-Limonene	C10H16	9.06	58378593.00	7.85
8	Eucalyptol	C10H18O	9.24	41987639.00	5.65
9	cis-Î²-Terpineol	C10H18O	10.95	7303608.00	0.98
10	trans-p-Menth-2,8-dien-1-ol	C10H16O	13.37	1138414.00	0.15
11	Terpinen-4-ol	C10H18O	15.10	6290008.00	0.85
12	trans-Dihydrocarvone	C10H16O	15.83	46013563.00	6.19
13	1,6-Dihydrocarveol	C10H18O	15.95	53792023.00	7.23
14	cis-Carveol	C10H16O	16.85	5529055.00	0.74
15	cis-3-Hexenyl isovalerate	C11H20O2	17.32	2411132.00	0.32
16	(-)-Carvone	C10H14O	17.98	376596019.00	50.65
17	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	C10H16O	18.16	1644285.00	0.22
18	Carvone oxide, cis-	C10H14O2	18.95	1828189.00	0.25
19	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.alpha.,5.beta.)-	C10H18O	20.11	4528392.00	0.61
20	.(-)-Dihydrocarvyl acetate	C12H20O2	20.95	28628231.00	3.85
21	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate	C12H18O2	21.25	2382921.00	0.32

Remark:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by

Name	Signature	Date
Gemechu Sorsa	[signature]	18/04/2019

Technical Signatory

Authorized by

Name	Signature	Date
Mulugeta Terefe	[signature]	18/04/19

Laboratory Manager

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JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Page 2 of 3	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name	Gebremichael G/hiwot	Test Report No	121	
Contact Person:	Gebremichael G/hiwot	Reported Date:	18/04/2019	
Sample Type:	Spearmint Leaves Essential Oil	Test Request No:	Not Specified	
Sample Source:	Not Specified	Tel:	+251 920 57 17 87	
Sample collected by:	Customer	Fax:	Not Specified	
Sample Collected Date:	10/04/2019	E-mail:	gimwot@yahoo.com	
Sample Received Date:	15/04/2019	Tested By:	LE-02	
Sample Condition	Normal	Date Tested:	16-17/04/2019	

S/N	Name	Formula	RT	Area	Area %
22	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	C10H14O	21.48	1555734.00	0.21
23	trans-Carveyl acetate	C12H18O2	22.31	4031102.00	0.54
24	(-)-.beta.-Bourbonene	C15H24	23.29	11757496.00	1.58
25	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	C15H24	23.54	8849058.00	1.19
26	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	C11H16O	23.69	4265066.00	0.57
27	Caryophyllene	C15H24	24.73	12091911.00	1.63
28	beta.-copaene	C15H24	25.13	1196803.00	0.16
29	Isogermacrene D	C15H24	25.70	1374486.00	0.18
30	cis-Muurola-4(15),5-diene	C15H24	26.42	1823749.00	0.25
31	Germacrene D	C15H24	27.21	4896946.00	0.66
32	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	C15H24	27.86	1354975.00	0.18
33	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	C15H24	28.40	1220313.00	0.16
34	trans-Calamenene	C15H22	29.04	2178191.00	0.29
35	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	C15H24O	32.24	2951503.00	0.40

Remark:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by			***Authorized by***		
Name	**Signature**	**Date**	**Name**	**Signature**	**Date**
Gemechu Sorsa	....	18/04/2019	**Mulugeta Terefe**	....	18/04/19
Technical Signatory			***Laboratory Manager***		

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JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Page 3 of 3	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name	Gebremichael G/hiwot	Test Report No	121	
Contact Person:	Gebremichael G/hiwot	Reported Date:	18/04/2019	
Sample Type:	Spearmint Leaves Essential Oil	Test Request No:	Not Specified	
Sample Source:	Not Specified	Tel:	+251 920 57 17 87	
Sample collected by:	Customer	Fax:	Not Specified	
Sample Collected Date:	10/04/2019	E-mail:	gimwot@yahoo.com	
Sample Received Date:	15/04/2019	Tested By:	LE-02	
Sample Condition	Normal	Date Tested:	16-17/04/2019	

S/N	Name	Formula	RT	Area	Area %
36	Caryophyllene oxide	C15H24O	32.51	6962763.00	0.94
37	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	C15H26O	33.34	1318800.00	0.18
38	Epicubenol	C15H26O	34.87	3696384.00	0.50
39	alpha.-Cadinol	C15H26O	38.15	4214409.00	0.57
40	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	C15H24O	40.05	1406900.00	0.19
41	n-Hexadecanoic acid	C16H32O2	46.59	1175498.00	0.16

S/N	Parameters	Test Methods
1	Essential Oil Profile	GC-MS

Remark:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by

Name	Signature	Date
Gemechu Sorsa	[signature]	18/04/2019

Technical Signatory

Authorized by

Name	Signature	Date
Mulugeta Terefe	[signature]	18/04/19

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APPENDIX C: Results of the chemical properties

Property	V1(ml)	V2(ml)	From eqn	value
AV	0.1		3.2	0.045
PV	32.9	33	3.3	0.67
SV	21.1	36	3.1	41.83
IV	42.5	18.9	3.4	17.8

Table c1

Analysis of variance table [Partial sum of squares]

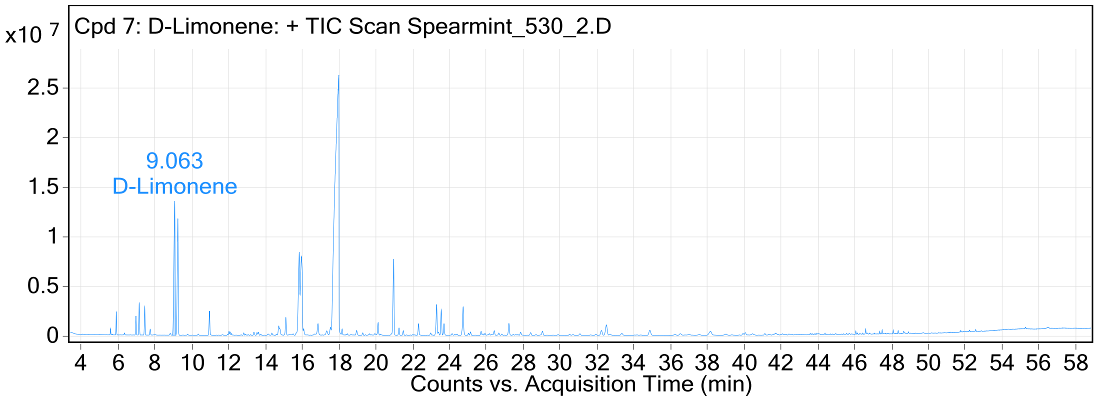
Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	0.29	13	0.023	12.03	< 0.0001	significant
A	*4.496E-003*	*2*	*2.248E-003*	*1.19*	*0.3344*	
B	*0.077*	*1*	*0.077*	*41.05*	*< 0.0001*	
C	*0.19*	*1*	*0.19*	*100.91*	*< 0.0001*	
B^2	*3.630E-004*	*1*	*3.630E-004*	*0.19*	*0.6679*	
C^2	*2.269E-003*	*1*	*2.269E-003*	*1.20*	*0.2925*	
AB	*1.211E-003*	*2*	*6.056E-004*	*0.32*	*0.7308*	
AC	*1.078E-003*	*2*	*5.389E-004*	*0.29*	*0.7559*	
BC	*0.013*	*1*	*0.013*	*7.08*	*0.0196*	
ABC	*4.467E-003*	*2*	*2.233E-003*	*1.19*	*0.3366*	
Residual	0.024	13	1.884E-003			
Cor Total	0.32	26				

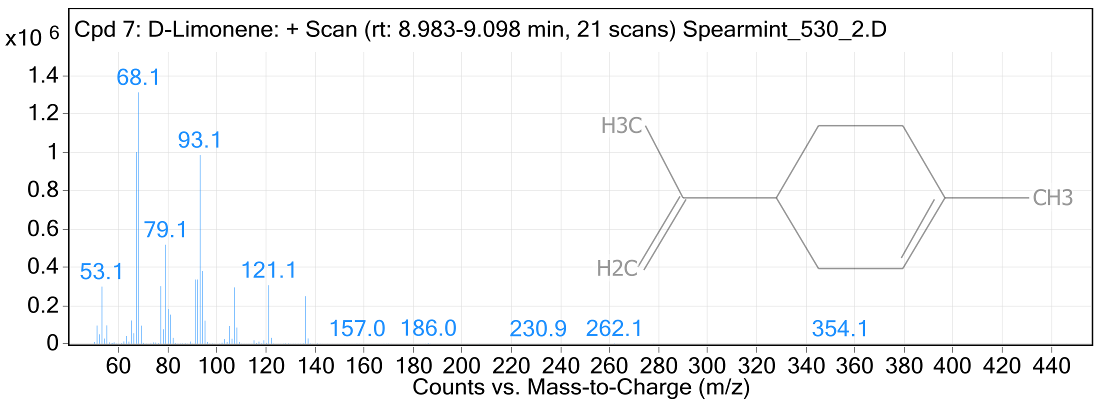
The Model F-value of 12.03 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

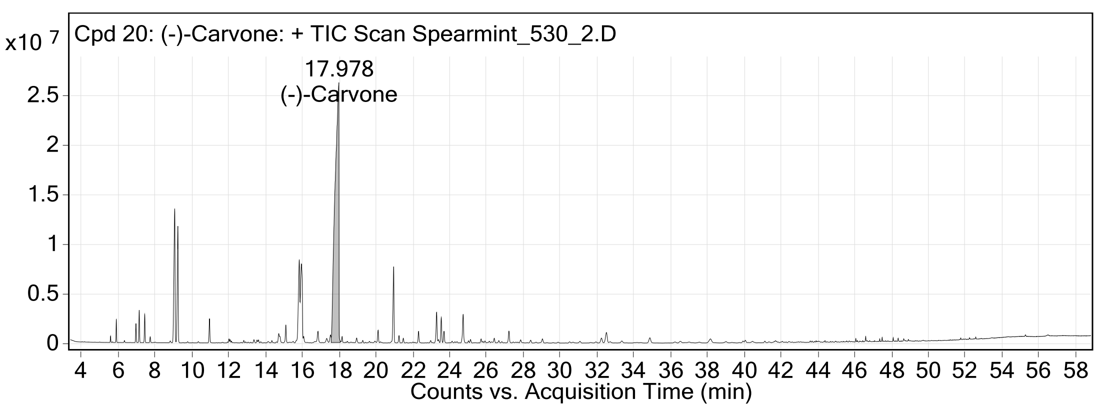
Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, C, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

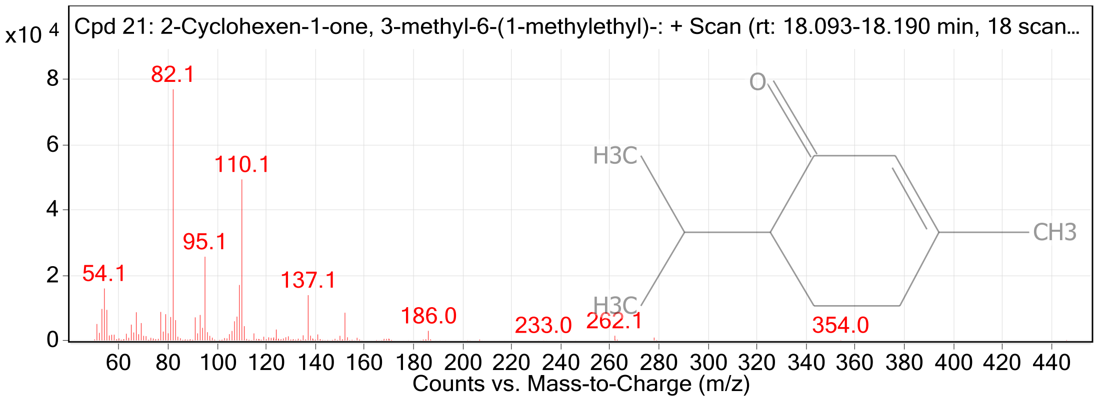
Table c2

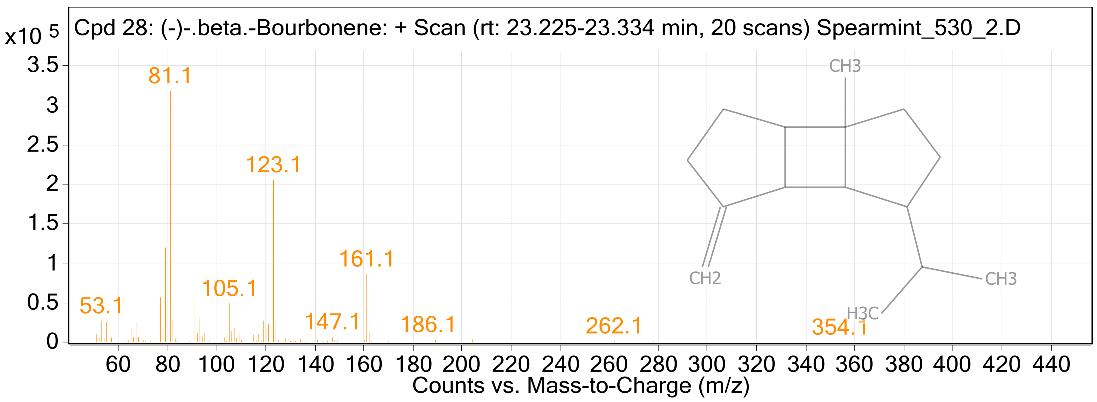
APPENDIX C: Qualitative Analysis Report








Cpd 21: 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-: + Scan (rt: 18.093-18.190 min, 18 scan...
x10 4
82.1
110.1
95.1
54.1
137.1
186.0
233.0
262.1
354.0
O
H3C
H3C
CH3
Counts vs. Mass-to-Charge (m/z)


Cpd 28: (-)-.beta.-Bourbonene: + Scan (rt: 23.225-23.334 min, 20 scans) Spearmint_530_2.D
x10 5
81.1
123.1
161.1
105.1
53.1
147.1
186.1
262.1
354.1
CH3
CH2
CH3
H3C
Counts vs. Mass-to-Charge (m/z)