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ABSTRACT

Peppermint oil is obtained from the leaves of the perennial herb, *Mentha piperita* L. and *M. arvensis* var. *piperascens* a member of the Labiatae family. This family includes many well-known essential oil plants such as spearmint, basil, lavender, rosemary, sage, marjoram and thyme. This is a well known and important medicinal plant widely used in several indigenous systems of medicine for various therapeutic benefits viz. analgesic, anesthetic, antiseptic, astringent, carminative, decongestant, expectorant, nervine, stimulant, stomachic, inflammatory diseases, ulcer and stomach problems. The present review is an up-to-date and comprehensive analysis of the chemistry, pharmacology, analysis, and uses of Peppermint oil.

❖ INTRODUCTION

The production of aromatic species to obtain essential oils has a great economic importance, mainly due to the increasing demand generated by food, cosmetics and pharmaceutical industries . Among many aromatic species, menthol mint has a great market demand, a consequence of its various applications in different sectors of industry. The genus *Mentha* belongs to the Lamiaceae family and comprises a large number of species, including *M. arvensis*. This specie is herbaceous; the composition of the essential oil varies a lot among the varieties, during the year and at different stages of its development, but is mainly composed of monoterpenes as menthol (70% - 90%), which is the major substance (see **Figure 1**). Essential oils are complex mixtures containing different classes of substances, mainly terpenoids and phenylpropanoids, which have diverse chemical structures that are part of special metabolism . Moreover, essential oils perform multiple roles for the plants, but can be involved mainly in species-specific ecological interactions For example, the important role in attracting pollinators, the inhibitor of herbivory, in protection of internal oxidation processes and light stress. On the other hand, others authors showed the action of essential oils and natural products on microorganisms of agricultural significance and on the seed germination. In this context, the search for new natural products becomes a valuable alternative to the conventional use of pesticides, used against cultivated plants diseases. The pathogenic fungi are microorganisms that cause economic damage directly in the agricultural production system, besides indirectly cause ecological problems, due to the use of pesticides for its control. The production of essential oils involves biosynthesis, transport, storage and degradation, each of these processes governed by genes and influenced by factors such as heredity, stage of growth and the environment.

Peppermint has traditionally been a major crop in the northwest and in 2010 California, Idaho, Oregon, and Washington grew a combined total of 56,700 acres of peppermint (Jenkins 2012). Extracted mint oil is sold through distributors and specialty oil formulators such as A.M Todd and I.P. Callison to major brand companies such as Wrigley and Proctor and Gamble, to be used in cosmetics and food products.

In order to obtain the essential oil, it must be extracted; with steam distillation being the most commonly used method for extraction of mint oil from peppermint plants. It uses steam to break apart the oil glands and transport the peppermint oil out of the mint. The two main components of

mint oil are menthone and menthol, which have boiling points of 207°C and 212°C, respectively (Sandborn 1929, C.R.C. 1971). Due to their large mole fractions the boiling point of peppermint will be somewhere within this region. The steam is able to remove the mint oil without reaching the boiling temperatures of menthone and menthol via Raoult's Law. Raoult's Law can only be considered valid when the system is ideal and all intermolecular forces are the same (Koretsky 2004). This case can be considered ideal because it is treated as a dilute system; the amount of mint oil in the mixture is considered to be approximately 1% mole fraction. At 1% we can assume the vapor pressure and boiling temperature will be very close to that of water.

The generation of steam is a very energy intensive process and the rising cost of natural gas and diesel has inspired research into possible ways to improve efficiency and yield, along with decreasing the carbon footprint. Additionally, due to a recent spike in diesel prices many farmers have been converting their boilers to natural gas. Natural gas is a much cleaner burning fuel and the conversion has only a 1-4 year payback. However, it is not clear if natural gas will remain at such a low price. The purpose of this research was to explore more sustainable options for the extraction process. Rocky Lundy, Executive Director of the Mint Industry Research Council, reported that 44% of carbon emissions from the entire process come from steam distillation (Gregory 2012). A continuous microwave extraction process has been proposed, which promises a greater efficiency and oil yield utilizing the more stable energy price of electricity. However the open ends of the microwave allows air to enter the mixture. The air insulates the steam and traps it as humidity resulting in a loss of product. An improved condenser design is necessary if SFME is adopted and put to use for the extraction of mint oil.

In SFME mint is exposed to microwave radiation via a microwave applicator. The microwaves increase the vibrational energy in the water molecules, this continued increase in energy causes the water to boil and change phase. SFME been shown to be able to remove up to 65% of available oils and take anywhere from 1.5-45 minutes. SFME in comparison to steam distillation can yield up to three times the amount of oil and due to shorter processing times reduce the cost of energy (Velasco 2007, Lucchesi 2004). One main difference between SFME and steam distillation is the source of the steam for the extraction. In SFME the steam is generated from water remaining in the leaves of the plants. This causes greater disruption of the surface of the leaves and is hypothesized to be one of the reasons more oil is extracted.

Scanning electron microscopy (SEM) pictures were taken before and after mint leaves were processed by the solvent free microwave method. The before and after SEM images are shown in Figures 2.51 and 2.52 respectively (Velasco 2007). The images show the peltate glandules have broken apart after the microwave process has occurred. Similar SEM images have been reported for essential oil extraction of cardamom seed. Researchers observed differences in solvent free microwave extraction from hydro-distillation, showing that cells are broken and damaged much more noticeably during SFME (Lucchesi 2007).

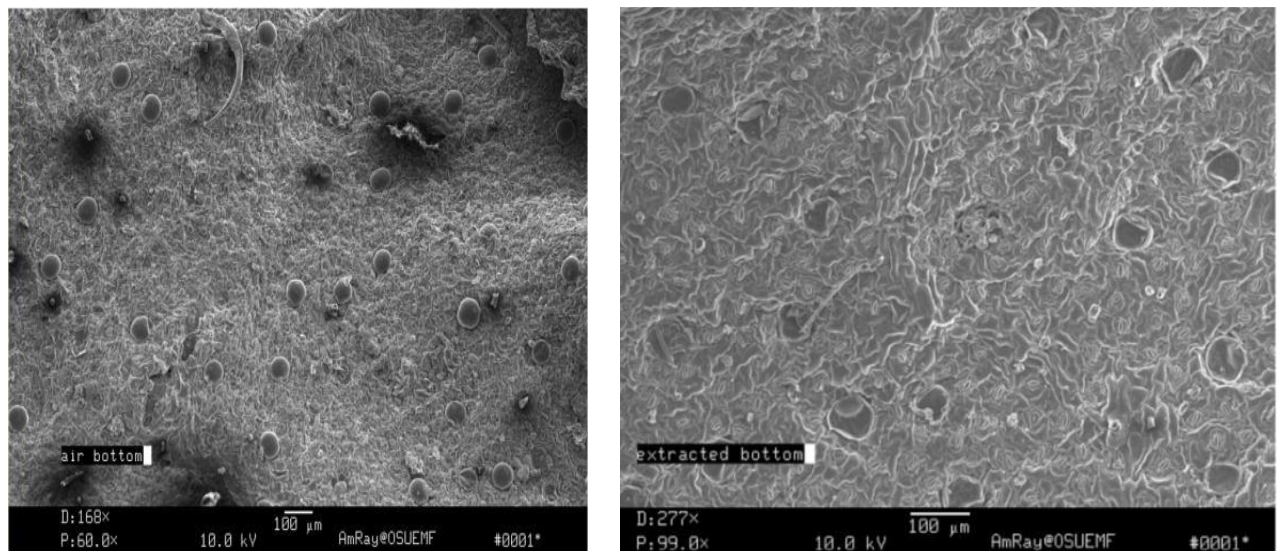


Figure 1: (left) SEM Peppermint leaf prior to microwave extraction. (right) SEM Peppermint leaf post microwave extraction (Velasco 2017).

❖ MATERIALS AND METHODS

3.1. Materials

For the experiment were obtained the following materials: alkane standard solution C8-C40 (Fluka, St. Louis, USA); Analytical standards (-)-limonene, (+)-menthofuran, eucalyptol, sabinene, (-)- α -pinene, (-)-menthone, α -terpineol, (+)-neomenthol, (-)-pulegone, (-)-menthol; the solvents dichloromethane, dimethylsulfoxide (Sigma-Aldrich, SP, Brazil); commercial seeds of lettuce, White Boston variety and tomato, Santa Cruz Kada variety (Isla Seeds Ltd., RS, Brazil); seedlings of menthol mint (*Mentha arvensis* L.) IAC 701 variety provided by Linax Corporation (Votuporanga, SP) and pure fungal cultures were obtained from mycology collection of the sectors of phytopathology of the Federal University of Viçosa (MG, Brazil).

3.2. Plant Material, Essential Oil Extraction and Analyses

Seedlings of menthol mint IAC 701 variety were propagated directly in definitive beds at Chemistry Department of Universidad Federal Rural do Rio de Janeiro (UFRRJ). The plants were grown for approximately 60 days, when young leaves (3rd to 5th node) and adult (6th to 8th node) were collected for extraction of essential oil by hydrodistillation (50 g of fresh plant material), with Clevenger apparatus, during one hour. The oil content (% w/w) was evaluated and the composition was analyzed by gas chromatography (GC) (Hewlett-Packard 5890 Series II, Palo Alto, USA) equipped with flame ionization detection and a split/splitless injector, in a split ratio of 1:20 was used to separate and detect the constituents in the essential oil. The substances were separated into the fused silica capillary column CPSil-8CB [30 m \times 0.25 mm (i.d.) \times 0.25 μ m (film thickness)]. Helium was used as the carrier gas at a flow rate of 1 mL \cdot minute⁻¹. The column temperature was programmed as follows: 60°C for 2 minutes followed by heating at 5°C \cdot minute⁻¹ to 110°C, followed by heating at 3°C \cdot minute⁻¹ to 150°C and finally followed by heating at 15°C \cdot minute⁻¹ until 290°C and holding constant for 15 minutes. The injector temperature was 220°C and the detector temperature was 280°C. The percentage of components in the essential oil was calculated from the relative area of each peak analyzed by flame ionization detector.

The gas chromatography coupled with mass spectrometry (GC-MS) was used for the essential oil analysis using a Varian Saturn 2000 (Palo Alto, CA). The flow of the helium gas carrier, the capillary column and the temperature conditions for the GC-MS analysis were the same as described for the GC. The temperature of the injector was 220°C and the temperature of the interface was 250°. Mass spectra were obtained with an ion-trap detector operating at 70 eV, with 40 - 400 m/z mass range and scanning rate equal to 0.5 scan·second⁻¹. The identification of the oil constituents was based on comparisons of their GC retention indexes and their mass spectra with authentic standards, NIST database (2008) and retention index (RI) [27]. The RI was obtained from the co-injection of an alkane standard solution.

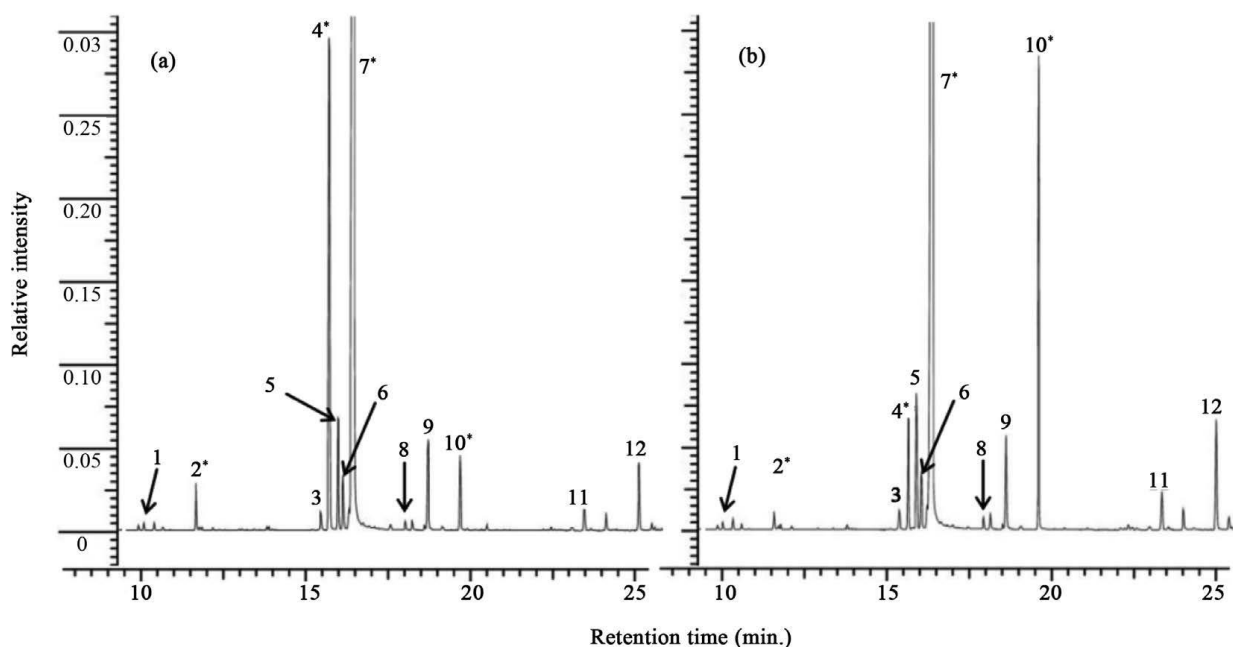
3.3. Description of the Experiment and Statistical

To evaluate the antifungal and antigerminative effects of menthol mint essential oil and same chemical substances isolated (–)-limonene, (+)-menthofuran, eucalyptol, sabinene, (–)- α -pinene, (–)-menthone, α -terpineol, (+)-neomenthol, (–)-pulegone and (–)-menthol were performed the following procedures. Antigerminative activity-seeds of tomato and lettuce were previously separated, 50 seeds per replicate, treated with a solution of dichloromethane (DCM) with 1.0 and 5.0 mg·mL⁻¹ of essential oil and their compounds isolated, respectively. The seeds were immersed in the solution until the total evaporation of the DCM, and then were transferred to Petri dishes containing filter paper and 3 ml distilled water. Finally, the dishes were sealed with plastic film and placed in the germination chamber with photoperiod of 12 hours and 25°C ($\pm 1^\circ\text{C}$). Two controls were prepared under the same condition, with DCM and the untreated seeds. At the end of the experiment, were considered as germinated seeds with healthy free leaflets and the radicle developed. Antifungal activity was utilized dilution method, in which the essential oils and isolated substances were diluted on potatoes dextrose agar (PDA) medium at 1.0 and 5.0 mg·mL⁻¹, respectively. To the dilution of the essential oils and chemical substances was used the dimethylsulfoxide (DMSO, 0.5% v/v). Approximately 5 ml of PDA medium containing the active substances were transferred to Petri dishes and, after solidification, were placed in the center of these dishes disks with 5 mm diameter containing propagative structures of *F. oxysporum*, *R. solani* and *S. rolfsii*. Two controls were prepared in the same conditions,

with DMSO (0.5%) and only the culture medium. To avoid the growth of bacteria was used in the culture medium gentamicin ($200\ \mu\text{g}\cdot\text{mL}^{-1}$), an antibiotic of broad spectrum. The dishes were placed in a thermostatic chamber at constant temperature of 25°C ($\pm 1^{\circ}\text{C}$) and were monitored daily the average diameter of the colonies in the two orthogonal directions until that controls reach $\pm 80\%$ of the total diameter of the plates. Plants of menthol mint were randomly collected in the center of three replicate blocks, until completing 50 g of young and adult leaves. Each treatment of antigerminative and antifungal activity test were composed of five replicates. The mean and confidence interval (CI) were calculated in Sigma Stat 2.03 (Chicago, USA). The graphics were created in Graph Pad Prism, version 5 (Graph Pad Software Inc., San Diego, USA).

3.4. Antifungal Activity

Table 2 shows that essential oil affected partially the development of the fungus *F. oxysporum*, *R. solani* and *S. rolsii*. Under these conditions the antifungal effect is characterized as fungistatic and may be caused by the synergism of substances of the essential oil.



❖ Experimental Setup

Mint hay is received via a standard mint tub into an in-feed conveyor unit with counter rotating vanes above the belt to produce a constant four inch thickness layer of mint hay for deposition on the microwave applicator conveyor. A spray bar enables the addition of water in the event the mint hay is deemed too dry for processing. It is then conveyed through a microwave choke unit and into the microwave applicator section. After entering the applicator, microwave energy (915 MHz) is applied to the mint hay and absorbed by the water in the hay. The microwave applicator system employed was a standard prototype testing unit from Industrial Microwave Systems, LLC (IMS) of Morrisville, NC. The unit selected included a 3-foot, 7-inch wide belt material application region. Roughly up to 150 Amperes are used during normal operation to produce up to 100 KW microwave power. A “circulator” is inserted between the generator and the transmission wave guide assembly. This unit directs reflected power (if any) that returns toward the generator into a termination device. The heated mint hay continues through a region within the applicator of slight negative pressure which is designed to withdraw the vapors to the condensing system. The hay then passes through another microwave choke unit to contain the microwave energy within the applicator region and then the hay is transported onto the open exit portion of the applicator belt. The microwave applicator belt then deposits the spent mint hay onto the out-feed conveyor belt which transports it to the waste receiving truck.

The mint oil, steam and air vapor mixture is drawn from the microwave applicator through an 8” round duct to a standard “DU-100” condenser and separator system manufactured by Newhouse, Inc. of Redmond, OR. The duct route included an eight foot vertical rise above the microwave applicator and a gradual incline down to the receiving end of the vertical tube and shell condenser, similar to that experienced from a standard mint tub to a condenser in a conventional system. To enable the vapor transport to the condenser, a variable speed blower was attached to the exhaust portal of the condenser to produce a modest negative pressure at the inlet end of the condenser.

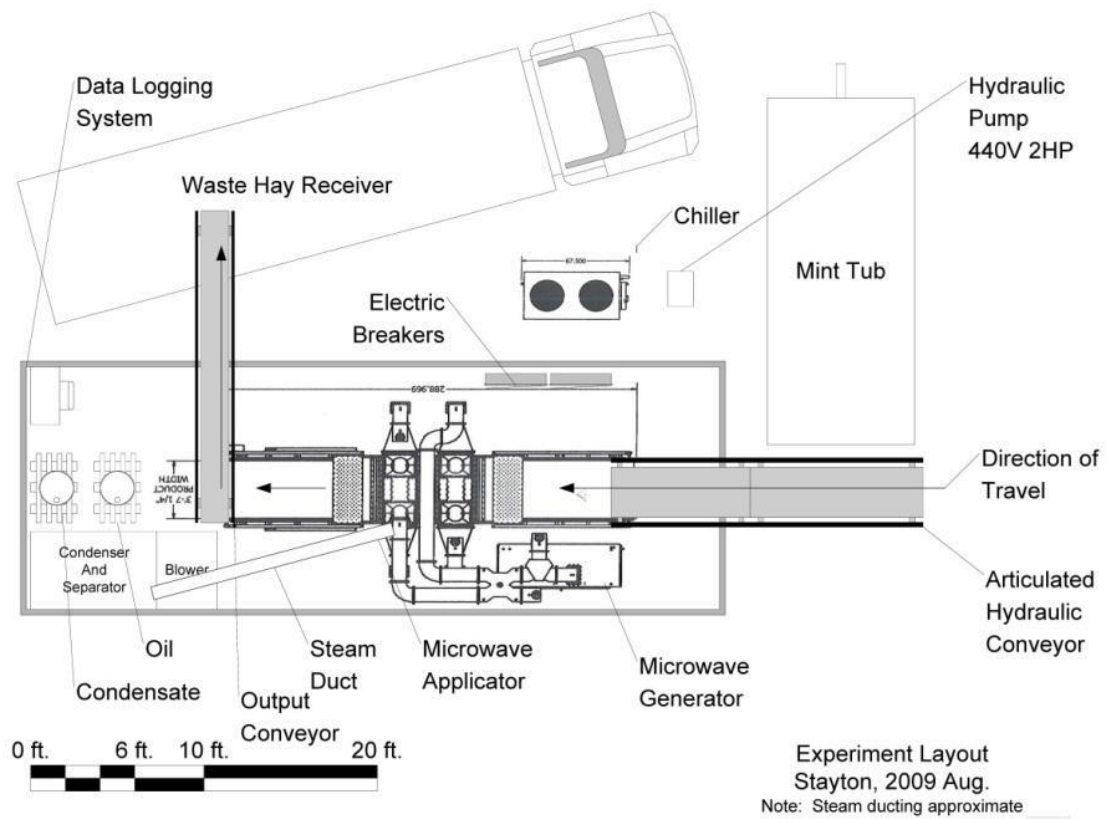


Figure 2: Experiment Layout (to scale)

This was deemed necessary as the vapor transported from the microwave applicator is a mixture of steam, mint oil vapors and air drawn in from the ends of the open conveyor belt containing mint hay. The variable nature of the exhaust blower was to enable total flow rate to be controlled based on the rate of steam condensation and hence negative pressure applied during the process when vapors arrived at the condenser. The exhaust air line then travelled from the building. The condensate line from the tube and shell condenser was attached directly to a standard “teepee” condenser of Newhouse design, identical to the other such units at the Butler Farm operation. From the separator, an oil transport line was sent to a barrel on a platform balance and the water separated was sent to another barrel on a separate platform balance.

In order to measure the specific conditions during the operation, a set of sensors, associated data logging equipment and software were assembled. Pressure transducers, thermistors and a vortex flow meter were ordered from Labjack® and Omega Engineering Inc.¹ Current sensors for measuring the energy demands of the blower, hydraulic conveyor belts, and microwave generator were obtained from Enercorp Instruments Ltd². A barrel scale for measuring condensate flow rate was purchased through Rice Lake Weighing Systems³. Analog signals of temperature, pressure, weight, flow, and current were wired into a Labjack® U3 PLC unit ordered from Labjack Corporation⁴ and interpreted with the software package, “DAQFactory⁵.” The flow meter was attached to the condenser cooling water input line and output both temperature and flow rate. Thermistors were installed on cooling water output, vapor input, and condensate output lines. Pressure sensors were placed at the vapor input and output streams to ensure that exit pressure was high enough to prevent condenser shell collapse. The Newhouse Tube and Shell condenser comes equipped with a thermistor sensor on the condensate line as well as a flow controller that increases coolant flow on demand from a set point. The microwave generator and applicator system had incorporated within it three IR sensors above the belt track in the applicator to allow monitoring of the hay surface temperature to take place. Power sensors on the microwave system enabled the measurement of forward, reflected and “pass through” power. Pass through power is based on energy that, for example, is not absorbed by the mint hay and continues along the applicator wave structure to a termination load. Above the exit site for spent hay from the applicator, an Infra-red detecting camera was imaging and observing the surface temperature of the exiting hay. It was believed that a hay exit temperature on the order of 105°C would be satisfactory in that the hay temperature would have exceeded the boiling point of water and yet not initiated combustion. Such was set and reproducibly accomplished with an input power of 45 KW and a belt speed of 3 fpm. Upon establishment of these operating conditions, large quantities of

steam and vapor were emitted within seconds from the mint hay samples while in the applicator and modest quantities continued to be emitted from the hay upon exit from the applicator region.

During the entire process, no mint oil was received in the separator unit. However, when the condenser line was disconnected from the separator, a stream of fluid was observed that had the distinct odor of pure mint oil. A small portion of this fluid was captured and analyzed via gas chromatography and the results are pictured in Figure 3. In essence, it appears that more Menthol and less Menthone were found in the oil extracted than in that from the normal process. A tube and shell heat exchanger was used to condense the steam and oil stream. However, the tube and shell was unable to successfully condense much of the mixture. It was determined that air was getting drawn into the stream with the steam and mint oil through the open ends of the microwave.

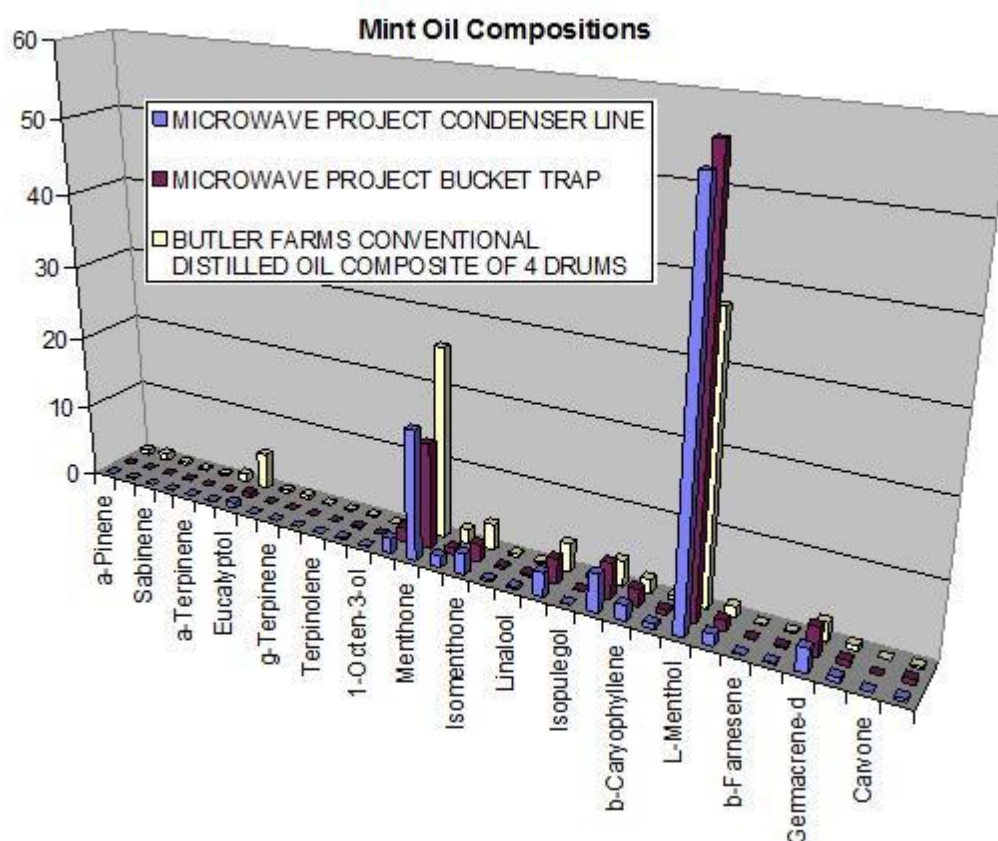


Figure 3: Recovered mint oil composition

It is known that in the presence of a noncondensable vapor, tube and shell condenser performance will be greatly reduced (Collier 1981). This is due to a gas boundary layer developing as condensation is formed along the condenser tube and it is this boundary layer which resists the steam from condensing (Seunguim 2006). It is more difficult to condense because the air acts as an insulator against heat transfer.

Microwave extraction could also change the way that farmers harvest their crop. Currently after being cut the plants must sit in the field to dry for several days to enable a chopping process to take place on the plant material. Research suggests that during this waiting time period no significant mass of oil is lost (Gershenzon 2000). However there is a significant risk of weather conditions affecting the oil in the plants. In addition, the composition of the oil is changed during this drying period. After drying the hay is then chopped and placed into large mobile trailers called mint tubs for processing. The microwave system requires higher moisture content in the mint hay than steam distillation during processing or ignition and combustion can take place. Table 1 shows some of the power ratings and belt speeds used for the microwave experiments.

❖ Important Of Mint Oil

1. It also has antibacterial, antiviral, anti-inflammatory, insecticidal, antispasmodic and carminative properties. The **health benefits of peppermint oil** include its ability to treat:
Indigestion: One of the oldest and most highly regarded herbs for soothing digestion is **peppermint**.
2. Its invigorating properties lend the **oil** its energizing effects. Used medicinally, **Peppermint** essential **oil** has been found to eliminate harmful bacteria, relieve muscle spasms and flatulence, disinfect and soothe inflamed skin, and to release muscle tension when used in a massage.
3. Peppermint essential oil is multi-purpose, earning the reputation of being one of the most versatile oils in the world along with Lavender oil.
4. The most active components of Peppermint essential oil are Menthol and Menthone, which are known to reduce pain and to invigorate, energize, and prevent the growth of harmful bacteria, respectively.
5. There are numerous applications for which Peppermint essential oil can be used, including cosmetics, aromatherapy, relaxing baths, and as a cleaning agent around the house.

❖ Uses And Applications

In a diffuser, Peppermint oil can help to enhance relaxation, concentration, memory, energy and wakefulness.

When used topically in homemade moisturizers, the cooling and calming effects of Peppermint essential oil can relieve sore muscles. Historically, it has been used to reduce itchiness and the discomfort of inflammation, headaches, and joint pains. It can also be used to relieve the sting of sunburns.

In a diluted massage blend or bath, Peppermint essential oil is known to relieve back pain, mental fatigue, and coughs. It boosts circulation, releases the feeling of having tired feet, relieves muscular pain, cramps, and spasms, and soothes inflamed, itchy skin among other conditions.

Well-known for its anti-microbial and anti-fungal qualities, a few drops of the oil can be added to homemade spray cleaners and spritzed on areas that are particularly in need of anti-bacterial care, such as bathrooms and kitchens. Peppermint makes an effective and natural deterrent for house pests and insects.





❖ Uses

- a treatment for a variety of conditions, including irritable bowel syndrome (IBS), nausea, and other digestive issues, as well as the common cold and headaches.
- a topical application for relief from itching, muscle pain, and headache.
- a flavouring agent in foods and in products such as mouthwashes.
- a fresh, pleasing scent added to soaps and cosmetic products.





CONCLUSION

For this experiment, an attempt was made to utilize as much of the traditional process equipment as possible to enable a comparison to be made for just the microwave applicator as a replacement for the essential oil extraction device. From the results, it is clear that a more radical approach is necessary to utilize the microwave system effectively in essential oil extraction. Much of the needed information to enable such a system to be constructed has been obtained from this experiment. The next steps for such a development involve an equipment design and development phase followed by a full-scale prototype. The logistics necessary to accomplish this project were daunting as it is a brand-new method of extracting essential oils from plants. The author wishes to thank the team and sponsors for the support of this opportunity to explore technology. An additional study was performed at IMS in North Carolina in December, 2009 utilizing 400 pounds of mint hay collected and frozen during this operation.

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