STUDIES ON MICROWAVE STEAM DISTILLATION PROCESS FOR EXTRACTION OF TULSI ESSENTIAL OIL

By

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KERALA, INDIA

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THESIS

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

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DECLARATION

We hereby declare that this project report entitled **"STUDIES ON MICROWAVE STEAM DISTILLATION PROCESS FOR EXTRACTION OF TULSI ESSENTIAL OIL"** is a bonafide record of research work done by us during the course of research and that the report has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

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CERTIFICATE

Certified that this project report entitled, "**STUDIES ON MICROWAVE STEAM DISTILLATION PROCESS FOR EXTRACTION OF TULSI ESSENTIAL OIL**" is a record of project work done by Ms. Manju M, Mr. Pradhymn Jawdhekar, Mr. Safwan Ali A C, Ms. Shahanas P under my guidance and supervision and that is has not previously formed the basis for the award of any degree, fellowship, associateship or other similar title, of any other university or society.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percentage
&	: And
/	: Per
>	: Greater than
BHT	: Butylated Hydroxy Toluene
cm	: centi meter
DMAPR	: Directorate of Medicinal and Aromatic Plants Research
et al.	: and others
etc.	: Etcetera
GHz	: Giga Hertz
g	: Gram
hr	: Hour
Ha	: Hectare
HS	: Hydro Steam
J	: Joule
K.C.A.E.T	: Kelappaji College of Agricultural Engineering and Technology
Kilo	: Kilo
Kg	: Kilo gram
MAE	: Microwave Assisted Extraction
MHz	: Mega Hertz

MSD	: Microwave Steam Distillation
M	: Meter
Min	: Minute (s)
Ml	: Milli Litre
mm	: Milli Meter
O	: *Ocimum*
RI	: Refractive index
SD	: Steam Distillation
S	: Second
SEM	: Scanning Electron Microscopy
SFE	: Supercritical Fluid Extraction
Syn	: synonymn
T	: Tonne
TNAU	: Tamilnadu Agricultural University
V	: Volt
W	: Watt
α	: Alpha
β	: Beta
μ	: Micro

Introduction

CHAPTER I

INTRODUCTION

Medicinal plants are richest bioresource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines.

Essential oils are the volatile components distilled from the aromatic plant materials which have some characteristic flavour and taste. Essential oils represent the essence or active components of plants due to the presence of aroma compounds which are oily in nature. They are also called as volatile or etheral oils as they evaporate when exposed to atmosphere at ambient temperature. These oils are used principally in perfumery and food flavourings. It can be used in variety of ways like natural additives in foods and food products, air fresheners, feed additives, cosmetics, deodorizers and active compounds in packaging materials. Essential oils possess high antimicrobial potential which led to its use as biocides and insect repellents and they are good natural sources of several bioactive compounds. Essential oils can serve as the alternative additives or processing aid as green technology. The rate of essential oil is determining by the amount of essential oil from different parts of plants which is different. The quantities of essential oils produced around the world vary widely. Essential oil production is the key source of exports for many countries, mainly in Africa and Asia. Essential oil export figures for Indonesia, Sri Lanka, Vietnam, and India are very high.

Several extraction methods are applied in the manufacture and extraction of essential oils, and the method employed is normally dependent on what type of botanical material is being used. Essential oil is generally extracted by distillation which can be defined as separation of components from the mixture of two or more liquids by virtue of difference in their vapour pressure. There are three main systems of distillation viz, hydro, steam and hydro steam distillation. Other processes include solvent extraction, absolute oil extraction, resin tapping and cold pressing.

Steam distillation is most used to produce many types of essential oils. The process is cheaper than other extraction processes. It will not use any solvent and can make it safer than other processes.

Steam distillation is not likely solvent extraction. In SD, the most volatile compounds of the aromatic material were carried by steam generated from water. Then the steam is chilled in the condenser to form the hydrosol. The essential oil is then collected effectively by draining the water. The measure of essential oil produced relies upon the length of distillation time, the temperature, and mainly, the type and quality of the plant material. The yield of essential oils from plants is between 0.005% and 10% usually (Chemat *et al*., 2013).

These existing conventional extraction methods may encounter loss of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects and toxic solvent residue in the extract. Low yields, long extraction time, high energy utilisation, high cost and environmental pollution are other deficiencies of these techniques. These deficiencies have prompted the thought of the utilisation of new innovative technique in essential oil extraction, which normally utilise less solvent and energy, like microwave assisted extraction, supercritical fluid extraction, ultrasound extraction, controlled pressure drop process, etc. These advanced innovations which developed gradually conquered the limitation of conventional methods, and enhanced the extraction efficacy.

Microwave energy has been advanced recently for the extraction of organic compounds from environmental matrices. Microwaves are a form of electromagnetic radiation that is the waves of electrical and magnetic energy moving together through space. The microwave energy is used to accelerate or mediate the extraction of essential oil with steam or water in order enhance the extraction process. With microwave heating of food material the internal heating of the water present within the material tends to the rupture of the odoriferous receptacles which releases the essential oil in it which is then evaporated by the in-situ water of the plant material. Then it passes out of microwave region to a condenser and the essential oil is collected. And it is evident that the microwave assisted extraction of essential oil enhances the process in terms both quantity and quality. The advantages of microwave assisted over other conventional process are high extraction efficiency, less extraction time and increased yield with quality of the extracted oil due to mild conditions. Beyond that the microwave energy is a key technology in achieving the objective of sustainable and green chemistry in commercial applications.

Holy Basil leaves (*Ocimum tenuiflorum*) also known as Tulsi is native throughout the world tropics and widely cultivating for its medicinal value. It is an erect, much branched sub shrub, 30-60 cm tall (Palla *et al*., 2012). In traditional use aqueous extract of *O.tenuiflorum* leaves is used for common cold and fever. Whole powder is also used for treating jaundice and alleviating blood pressure and the chemical constituents present are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, Linalool, and β-caryophyllene (Palla *et al*., 2012). It is an aromatic plant in the family of *Lamiaceae.* It is also native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian Tropics. Tulsi is cultivated for medicinal purposes and also for its essential oil. (Mohammad *et al*., 2016). *O.tenuiflorum* has been described as the queen of medicine and the mother of medicine of nature due to its medical qualities (Singh *et al*., 2012). The Tulsi essential oil is also used as an edible coatings on fruits like guava and amla (Steffi *et al*., 2018). *Ocimum sanctum* oil was evaluated for its antimicrobial activity to use it as natural preservative for fermented dairy products and its compatibility with starter cultures (Santosh *et al*., 2016).

Mostly the tulsi essential oil extracted using distillation process where fresh or dried leaves are taken in a round bottom flask with water and heated, the oil and water mixture is condensed by running cold water and the oil is collected. Some studies have shown utilizing microwave to extract essential oil which reduces the extraction time and the quality of the oil is also improved so the microwave assisted extraction is explained as efficient method when compared to the conventional processes. In this context it may be noted that such optimization studies pertaining tulsi essential oil has not been found reported.

Taking the above facts into consideration, this research entitled **"Microwave assisted steam distillation process for extraction of Tulsi Essential oil"** was undertaken with the following objectives:

1. Evaluation of the system developed towards extraction of Tulsi oil, and optimisation of the process parameters.

2. Characterisation of the microwave steam distilled Tulsi oil in comparison with conventional distilled oil.

Review of literature

CHAPTER II

REVIEW OF LITERATURE

This chapter deals with the review of research work reported on the benefits of Tulsi and Tulsi oil. Reviews on application of microwave technology in the extraction of essential oil and advantage of microwave assisted over conventional process.

2.1 TULSI

Ocimum tenuiflorum (syn. Ocimum santum) is an aromatic plant popularly known as tulsi (Hindi), holy basil or sacred basil (English) is the most sacred herb among the Hindhus in India. *O.tenuiflorum* is an erect, tall (30-60cm), aromatic, sub-shrub with hairy sub-quadrangular branches. Leaves are simple, green or purple, ovate, elliptic-oblong, obtuse or acute, usually slightly toothed with entire or sub-serrate or dentate margins, pubescent on both sides, dotted with minute glands and slender hairy petioles. The aerial parts possess glandular hairs on stalked and sessile glands which secrete volatile oils (Pavan *et al*., 2015).

2.1.1 Area and Production

In India, basil is cultivated over an area of 25000 ha and it accounts for annual production of about 250-300 T of oil. *O.tenuiflorum* has widest distribution which covers Indian sub-continent, ascending upto 1800 m in the Himalaya and in Andaman and Nicobar Island. This plant can occupy a wide range of habitat. It flourishes well under fairly high rainfall and humid conditions. Long days and high temperatures have been found favourable for plant growth and oil production. It can grow up to an altitude of 900 m. The plant is moderately tolerant to drought and frost. The plant can be grown under partially shaded conditions but with low oil content (DMAPR, 2014).

Ocimum are well represented in the warmer part of both hemispheres from sea level to 1800 m. The main centers of diversity are Africa, South America and Asia. Different species are well distributed over tropical countries in these continents. Of the 160 species of *Ocimum* is cultivated for atleast 3000 years by

Europeans and Asians for the folklore and religious rituals and got established wherever they migrated with extreme variation in populations. It is grown and distilled for oil in France, Italy, Bulgaria, Egypt, Hungary, South America Comora Islands, Thailand, India, Haiti and Gautimela (Joy, 2007).

The plant is sufficiently hardy and it can be grown on any type of soil except the ones with highly saline, alkaline or water logged conditions. However, sandy loam soil with good organic matter is considered. The crop has a wide adaptability and can be grown successfully in tropical and sub-tropical climates. Long days with high temperature have been found favourable for plant growth and oil production (TNAU AGRITECH PORTAL, 2021).

2.1.2 Varieties

The major constituents of tulsi oil varies with the species. The different species of tulsi and its habitat and major constituents of oil is given in Table 2.1.

Table 2.1: Classification of different *O.* species (Horticulural Sciences, 2007).

Species	Habit	Major constituents of oil
O.basilicum L.	Herb	Methyl chavicol, methyl cinnamte, eugenol
O.americanum	Herb	Methyl chavicol, citral
O.tenuiflorum	Shrub	Eugenol
O.gratissimum	Shrub	Eugenol
O.canum Sims	Herb	Linalool, Methyl chavicol, methyl cinnamte, eugenol
O.kilimandscharium	Shrub	Camphor
O.viride Wild	Shrub	Thymol
O.micranthum	Shrub	Elimicin, eugenol, methyliso eugenol
O.carnosumck	Shrub	Eugenol

2.1.3 Tulsi essential oil

Essential oils, also known as essences or volatile oils, are substances biosynthesized by living organisms. They can be liberated from the material which contains them by distillation, pressing or extraction with a suitable solvent. They have found uses in fragrance, food, cosmetic and pharmaceutical industry (Springer *et al*., 2007). The composition of the essential oils varies according to the region they are cultivated in and to the climate conditions (Nampoothiri *et al*., 2012).

Tulsi are mostly cultivated for its essential oil which has broad pharmaceutical and industrial uses. Various factors such as cultivars type, chemotypes, ecotypes, transplanting date, method of drying, distillation type and others affect tulsi essential oil content and composition. (Bowes *et al*., 2004). The major constituents of tulsi essential oil are given in Table 2.2. It possesses the pleasant odour characteristics of the plant with an appreciable note of clove. Chemotype of *O.tenuiflorum* containing methyl eugenol as a major and minor constituent of essential oil has been reported from India and Thailand. Recently *O.tenuiflorum* contains higher essential oil concentration which is rich in methyl eugenol (>70%) has been isolated. Methyl eugenol is used widely in perfume compositions of the carnation type. It is also used as a flavouring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, icecream. As a flavouring agent it has spicy ginger like undertones and its odour is musty-tea like warm and mildy spicy (Kothari *et al*., 2004).

Table 2.2: The major constituents of *O.tenuiflorum* essential oil (Journal of essential oil research, 2014)

Compounds	Percentage (%)
Eugenol	34.3
β-caryophyllene	23.1
β-elemene	18
Caryophllene oxide	3.8
Thymol	2.4
(Z)-α-bisabolene	2.2
α-humulene	2.0
Elemol	0.9
Borneol	0.3

Tulsi leaves contain a bright yellow volatile oil which is useful against insects and bacterial. The oil is reported to posseses anti-bacterial properties and acts as an insecticide. It inhibits the in vitro growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var *aureas* (TNAU AGRITECH PORTAL, 2021).

2.1.4 Benefits of tulsi

O.tenuiflorum was reported to have anti-cancer, antimicrobial, antiseptic, antispasmodic, antifungal, antiviral, anti-inflammatory, analgesic and immune-stimulatory properties (Palla *et al*., 2012).

2.1.4.1 *Antimicrobial activity*

Sermakkani (2011) reported that antimicrobial activity of hexane, acetone and ethanol extracts of leaves of *O.tenuiflorum* by disc diffusion method against certain gram-positive and gram-negative bacterial pathogens and some fungus. The acetone extracts showed a wide range of antibacterial activity against bacterial and fungal pathogens than the hexane extract, whereas ethanol extract where slightly lower antimicrobial activity than acetone extract. The preliminary chemical test performed in all extracts showed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins.

2.1.4.2 Antioxidant activity

Balaji *et al*. (2011) reported the antioxidant activities and total phenolic assay in methanolic extracts of stem and leaves of *Ocimum tenuiflorum* by using BHT and ascorbic acid as standard antioxidant. Total phenolic content was estimated in both extracts and leaf extract show more activity almost 71 % higher. The superoxide anion scavenging activity is also more for leaf that is 81 % more. The carotenoid and ascorbic acid content is also more in leaf than stem.

2.1.4.3 Anti-cancer activity

Gajula *et al*. (2010) reported the anti-cancer activity in leaf powder of *O.tenuiflorum* in induced colon tumours in fisher 344 male rats. Carcinognesis was induced by subcutaneous administration of azoxymethane (16mg/kg body weight in saline) at 7th and 8th week. Tumours/tumour bearing rat ratio was reduced by 78% in rats. The extracts showed higher chemoprotective agent.

2.1.4.4 Anti-inflammatory

Rosmarinic acid also is a good source of anti-inflammatory along with being an antioxidant. Pegnin is one more compound available in compositin of serving the same function. Apart from these two, the most important anti-inflammatory driving force in tulsi is eugenol. It is main ingredient responsible for controlling the blood sugar levels in the body. It rigs the beta cell function of the pancreas and as a result augments the insulin secretion (Debjit, 2010).

2.1.4.5 Larvicidal activity

Rushikesh *et al*. (2010) reported the larvicidal activity in hydro-distillated oil obtained from fresh leaves of *O.tenuiflorum* in *Aedes aegypti* Instar III mosquito larvae. The volatile oil showed IC50 value of about 291.29 ppm the maximum activity showed at a concentration of 500 µg/ml about 64 %. Thus the essential oil was being very successful in preventing mosquito borne diseases such malaria, dengue, etc.

2.2 CONVENTIONAL METHOD OF EXTRACTION

Essential oils are commonly extracted by distillation, mostly hydro distillation and steam distillation. Other processes include expression, solvent extraction, cold pressing etc. In hydro distillation method, the herbage is packed in a vessel which is halfway loaded with water and heated by direct fire without an external boiler. This method is less proficient, however the unit is simple and less expensive. SD utilises an external boiler to produce steam which is to be brought into the chamber. In spite of the fact that the technique is more effective, it includes higher capital cost, yet the quality of oil obtained is superior. Thus the most usually utilised method is steam distillation.

The SD method involves placing of plant part, from which essential oil to be extracted (which can be either fresh or dried), into a vessel. Then, pressurised steam is allowed to concentrate in the area, where the items are placed, and allowed to saturate. After saturation, the essence (volatile aroma compounds) of plant part is sweated out due to bursting of cell walls and passes through a condenser along with steam. Essential oil can be collected by separating the hydrosol.

The main shortcomings of these conventional methods include low yields, long extraction time, high energy consumption, high cost, thermal and hydrolytic degradation and environmental pollution (Tongnuanchan and Benjakul, 2014). These inadequacies prompted new innovative techniques in essential oil extraction. Modern technologies which grew continuously conquered the limitation of conventional methods, and upgraded the extraction efficacy. Microwave assisted extraction is one of such techniques on the basis of interaction between water in the plant material and microwaves generated by the energy source. Contrasted with regular techniques microwave assisted extraction has high extraction rates, reduced energy consumption and more environment friendliness.

Yonei *et al*. (2003) were studied that the methods used for extraction of ginger oil are hydro distillation, steam distillation, H-S distillation, SFE-Co_2, solvent extraction, and microwave extraction as a recent technique; the oil yield, extraction time, and quality of oil extracted from each method differ significantly and dried rhizome was used in all the methods

2.3 MICROWAVES

Datta *et al*. (2000) stated that microwaves are the electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz with a corresponding wavelength ranging from 1 m to 1 mm. Domestic microwave appliances operate generally at a frequency of 2450 MHz, while industrial microwave systems operate at a frequency of 915 MHz and 2450 GHz. Microwaves are coherent and polarized in contrast to visible waves (apart from lasers). They obey the laws of optics and can be transmitted, absorbed or reflected depending on the type of material.

2.3.1 Dielectric properties

The ability of a material to convert microwave to heat can be understood by knowing its dielectric properties. The dielectric properties are mainly affected by the operating temperature and the microwave frequency used. Based on the microwave absorption, materials are classified into (i) absorbers or high dielectric loss materials which are strong absorbers of microwave (ii) transparent or low dielectric loss materials where microwave passes through the material with little attenuation and (iii) opaque or conductors which reflect the microwaves. Hence, a knowledge of dielectric properties is necessary to differentiate the materials into the above three categories (Metaxas *et al.*, 1983).

Apart from the dielectric properties and the penetration depth, other factors which affect microwave food processing are microwave oven design (oven size and geometry), microwave frequency, placement of food material inside the oven, moisture content, density, composition, load, shape and the size of food materials (Icier *et al.*, 2004). In general, amount of moisture or water content in a food material plays a deciding factor in determining the dielectric properties of the food material, since water is a good absorber of microwaves.

2.3.1.1 Factors affecting dielectric properties of food materials

Microwaves are not absorbed by the material due to its electronic or atomic polarization, however, they might be absorbed owing to its dipole or ionic polarization. Dipole polarization is significant at frequencies above 1 GHz while ionic losses are predominant at frequencies below 1 GHz (Ryynanen, 1995). The dielectric constant of pure water decreases slightly with frequency. Similarly, the dielectric loss increases with increasing frequency for moist foods. The dielectric properties of food materials are mainly determined by their chemical composition and to a less extent of physical structure. Generally, food material consists of a mixture of organic material, water and salt. The dielectric loss at a particular frequency increases with the addition of salt. Salt solutions act as conductors in presence of the electromagnetic field, hence decrease in the permittivity and increase in the dielectric loss factor was observed by Icier and Baysal (2004). The dielectric property of water varies depending on whether it is in free or bound state. In the presence of an electric field, the polar molecules of water in free state orient more freely than those of bound water. For high water content frozen materials, the dielectric properties might increase with an increase in temperature in the melting zone. During runaway heating of frozen and thawed foods, the warm part gets rapidly heated and at the same time there are still some ice left in the food material and hence poses non-uniformity issues (Ryynanen, 1995). The dielectric characteristics of the food materials may also vary with their particle size, structure and density of the material. Dielectric properties are also affected by the apparent density of the air-particle mixture of a granular or particulate material (Icier and Baysal, 2004). The dielectric properties of food materials such as bread, flour, fruits and vegetables depend mostly on their water content. Although, dielectric constant and dielectric loss values are generally low for fats and oils, an increase in dielectric loss with temperature can also be observed (Icier and Baysal, 2004). The variation of dielectric properties with temperature and microwave frequencies was investigated for solutions containing salt, sugar and carboxymethylcellulose (Coronel *et al*., 2005). Based on the results, it was concluded that CMC does not have any significant effect on the dielectric properties whereas it has an effect on the viscosity. For sugar solutions, dielectric constant increases with the temperature and the sugar concentration. However, the dielectric loss factor decreases with sugar concentration due to non-polar nature of sugar, however it was also found that the dielectric loss factor increases with temperature.

Thus, sugar, salt and CMC can be used to mimic the dielectric properties and rheological properties of the food product to be processed (Coronel et al., 2005). For natural honey with 18% moisture content, the dielectric loss increases with temperature for frequencies above 1 GHz. The dielectric loss of honey was found to increase with water content at low frequencies due to ionic conduction (Guo *et al*., 2011). Boldor and co-workers (2004) investigated the dielectric properties of in-shell and shelled peanuts at various densities, temperature and moisture content over a frequency range of 300 to 3000 MHz. At microwave frequencies of 915 and 2450 MHz, the dielectric properties were found to be dependent on temperature for low moisture content samples. On the other hand, at higher moisture contents, the dielectric properties were found to be less significant on temperatures (Boldor *et al*., 2004)

2.3.2 Applications of microwave in food industry

2.3.2.1 Microwave cooking

Cooking is one of the major applications of microwave. There are numerous reports on the baking of bread and cooking of rice and meat using microwaves. In many cases, a comparison is made between microwave cooking and traditional cooking. In bread baking process, it is essential to obtain browning and good texture at a fixed moisture level (Icoz *et al*., 2004).

Conventional baking using hot air provides suitable color and texture. In microwave baking, sufficient brown color on the surface of breads and crust formation were not possible. During microwave heating, the air surrounding the food product is cold and water evaporating from food gets condensed on contact with cold air, which results in the lack of crispness of food product. Susceptors were placed at the bottom of the sample to provide crust formation and surface browning of the food product. Note that, susceptors are microwave absorbing materials, which convert microwave to heat and supply the heat to the weak microwave absorbing materials by means of conduction and radiation. The color measurements were carried out using a chroma meter as L*, a* and b*. L* is a measure of lightness, a* is a measure of greenness to blueness while b* is a measure of redness to blueness. Lightness values were found to decrease with baking time and temperature which indicated that the color of the sample became darker. The results showed that zero order kinetics can explain the change in lightness using microwave. Browning was not observed without the help of susceptors (Icoz *et al*., 2004).

2.3.2.2 Microwave blanching

Blanching is generally used for colour retention and enzyme inactivation, which is carried out by immersing food materials in hot water, steam or boiling solutions containing acids or salts. Microwave blanching of herbs such as marjoram and rosemary was carried out by soaking the herbs in a minimum quantity of water and exposed to microwaves (Singh *et al*., 1996).

Microwave blanching was observed for maximum retention of colour, ascorbic acid and chlorophyll contents than that of water and steam blanching. Microwave blanched samples were found to have better retention of quality parameter than that of microwave dried samples without blanching (Singh *et al.*, 1996).

Similarly, water-assisted microwave treatment of fresh jalapeno peppers and coriander foliage were found to have effect against the pathogenic bacterium Salmonella typhimurium which resulted in the reduction 4-5 log cycles of microbial population (Vega-Miranda *et al*., 2012).

2.3.2.3 Microwave drying

Microwave drying has the advantages of achieving fast drying rates and improving the quality of some food products. The energy absorption level is controlled by the wet products which can be used for selective heating of interior parts of the sample containing moisture and without affecting the exterior parts. Microwave drying is considered very useful during falling rate period. During falling rate period the diffusion is rate-limiting, resulting in the shrinkage of the structure and reduced surface moisture content. However, in microwave drying, due to volumetric heating the vapors are generated inside and an internal pressure gradient is developed which forces the water outside. Thus shrinkage of food materials is prevented in microwave drying. Microwave energy combined with other drying methods can improve the drying efficiency as well as the quality of food products which is far better than that achievable by microwave drying only or by other conventional methods only (Zhang *et al*., 2006).

Microwave dried parsley leaves retained the colour and the change in microwave power level did not affect the colour parameters (Soysal, 2004). In drying of garlic cloves, microwave assisted air drying achieved a drying rate 80-90% better than that of conventional air drying, and the product of microwave assisted drying exhibited superior qualities (Sharma and Prasad, 2001).

2.3.2.4 Microwave pastuerizatuion

Microwave pasteurization of pickled asparagus achieved the required temperature for pasteurization twice as fast as (15 min for 1 kW and 9 min for 2 kW) conventional heating (30 min). The thermal degradation of asparagus was more when it was subjected to conventional treatment compared to when it was subjected to microwave heating (Lau and Tang, 2002).

2.4 MICROWAVE HEATING

Microwaves are electromagnetic waves with the frequency varies from 300 MHz to 300 GHz. Microwaving has been enormously applied in the field of food Processing such as drying, heating or cooking, pasteurization and preservation of foods (Chandrasekaran *et al*., 2013).

Microwave drying has many advantages, including lower shrinkage, lower bulk density, and higher rehydration ratio, dehydration rate and energy saving than traditional drying (Aydogdu *et al*., 2015). Microwave heating or cooking can retain high levels of bioactive components, antioxidant activity and attractive color of vegetables, when cooking without water or with a small amount of water (Akdaş *et al*., 2016). Microwave sterilization can not only effectively reduce the potential microorganisms in food to Ensure food safety, but can also inactivate the enzyme to maintain the nutrition of food (Chen *et al*., 2016).

2.4.1 Principle of microwave heating

Datta *et al*. (2000) stated that, the phenomenon of materials to absorb microwave energy and transform into heat is the main reason for microwave heating of materials. The two important mechanisms by which microwave heating of food materials mainly occurs are ionic polarization and dipolar rotation. Water has dipolar nature and dielectric heating is due to the presence of water or moisture in a given material. When an oscillating electric field is incident on the food materials, the water molecules which are permanently polarized dipolar molecules try to realign in the direction of the electric field. As the microwave frequency is very high about 2450 MHz, the water molecules vibrate 2450 million times per second which causes internal friction in between the molecules. This friction between the molecules leads to volumetric heating of the material.

Microwave radiation interacts with dipoles of polar and polarizable materials. The coupled forces of electric and magnetic components change direction rapidly (2450 MHz). Polar molecules try to orient in the changing field direction and hence get heated. In non-polar solvents without polarizable groups, the heating is poor (dielectric absorption only because of atomic and electronic polarizations). This thermal effect is practically instantaneous at the molecular level but limited to a small area and depth near the surface of the material. The rest of the material is heated by conduction (estandau *et al*., 2013)

Thus, large particles or agglomerates of small particles cannot be heated uniformly, which a major drawback of microwave is heating. It may be possible to use high power sources to increase the depth of penetration. However, microwave radiation exhibits an exponential decay once inside a microwave absorbing solid (Veggi *et al*., 2017).

In microwave heating process, energy transfer occurs by two mechanisms: dipole rotation and ionic conduction through reversals of dipoles and displacement of charged ions present in the solute and the solvent. In many applications these two mechanisms occur simultaneously. Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied, and the resistance of the solution to this flow of ions results in friction that heats the solution. Dipole rotation means realignment of dipoles with the applied field. At 2450 MHz, which is the frequency used in commercial systems, the dipoles align and randomize 4.93×109 times per second and this forced molecular movement results in heating (Veggi *et al*., 2012).

2.7 MICROWAVE ASSISTED EXTRACTION

Microwave assisted extraction is the treatment of plant material with microwave irradiation during extraction to enhance the recovery of secondary metabolites and aroma compounds. The forced heating of water in core of material may cause liquid vapourisation within the cells, which may lead to the rupture of cell walls and/or plasma membranes.

Movaliya (2017) claimed that MAE has been recognized as a technique with several advantages over other extraction methods, such as reduction of costs, extraction time, energy consumption, and Co_2 emissions. MAE technique is a green technique which is highlighted by increased extraction yield, decreased time and solvent consumption, moreover the reproducibility is better. This extraction method is rapid compared to conventional method like hydrodistillation, soxhlet extraction. MAE technique produced more oxygenated compounds, is more cost effective and environmental friendly. These results indicate that cinnamon oil extracted through MAE method exhibit better properties especially in terms of quality when with conventional hydrodistillation technique. It also suggests that MAE is suitable for extracting volatile oils from different plant material without necessarily causing any adverse change to the chemical composition of the oil.

Racoti *et al*. (2017) stated that the lower the water contents from the plant material, the better the MW penetration depth. For oven-dried ginger, the penetration depth is 40 times better than for fresh ground ginger root. Stirring ensures even exposure of the plant material to the MW action and increases the yield. A maximum energy input must be determined and not exceeded in order to avoid degradation of the plant material which was determined in the present work as 0.40 kWh/kg.

The use of MAE in the isolation of herbal essential oil is an interesting alternative that provides more effectiveness than other processes (Bousbia *et al*., 2009).

Under microwave irradiation, high-frequency electromagnetic wave can quickly penetrate the liquid medium to reach the inside of material, and convert into heat to increase the temperature inside the cells to evaporate water, which can produce pressure on the cell walls. Accordingly, the cell breaks down and its components are released when the pressure exceeds the maximum that the cell walls can with-stand (Mandal *et al*., 2007). As reported, the extension of microwave irradiation time can enhance the absorption of microwave energy, and further improve the dissolution of Essential oil (Hosseini *et al*., 2016).

Taghi *et al*. (2008) compared conventional and MAE method for obtaining essential oil from *Thymus vulgaris*. Dragovic-Uzelac *et al*. (2012) employed MAE to get polyphenols from wild sage and Kahriman *et al*. (2012) obtained essential oil from Vicia herb using hydro and microwave distillations.

Kusuma *et al*. (2016) conducted a study on extraction of essential oil from the orange peels (*Citrus auranticum* L.) by microwave steam distillation. The outcomes were contrasted SD in respect of extraction yield, time of extraction, chemical composition, and quality of the essential oils. MSD was found better with good extraction yield and extraction time (140 min for MSD, and 7 h for SD). Scanning electron microscopes (SEM) of orange peel undergone SD and MSD revealed that essential oil glands ruptured rapidly with MSD process than that of SD. Composition of extracted essential oil, analysed by Gas chromatography–mass spectrometry, was found similar in both method and did not indicate any adverse effect by microwave application. They found MSD is an alternate technique to extract orange essential oil which is used for fish growth promoter.

Sagarika *et al*. (2016) carried out experiments on microwave assisted extraction of nutmeg mace essential oil, compared it with conventional hydro-distillation and the quality characteristics of the essential oil were determined. Physical quality characteristics such as refractive index, specific gravity, solubility and color of essential oil were found to be similar in both methods whereas the main chemical constituent myristicin was found to be slightly higher in microwave assisted process than the hydro distillation method.

Claudia (2017) developed a microwave assisted steam distillation system for the extracting lemongrass essential oil. The result showed that an increase in essential oil yield was observed with increase in the bulk density and extraction time. The extraction was improved by increasing the microwave power 280 – 420 W. Total energy consumption and temperature of extraction enhance with increase in microwave power and time of extraction the process parameters were found to have insignificant effect on the physical quality characteristics of the oil extracted through microwave steam distillation and steam distillation. The optimized operating conditions of bulk density microwave power and time of extraction for lemongrass essential oil in microwave steam distillation where found to be of 0.375 gcm $^{-3}$, 420W and 30 min.

From the review of literature it is understood that MAE could be effectively used for extraction of essential oil. The main advantage of using microwave energy is it significantly increases the speed of the processes and reduces the thermal gradients and also solvent free environment friendly process. The essential oil from many plant materials were successfully extracted using microwave energy, but the studies pertaining to tulsi oil extraction using microwave assisted steam distillation has not been found reported. With this background, the present study anticipates development of a system for microwave assisted steam extraction of tulsi oil and optimization of the process parameters leading to high extraction efficiency and quality of the extracted oil. Such a study could produce tulsi oil of high quality and quantity and reduce operational costs.

Materials and methods

CHAPTER 3

MATERIALS AND METHODS

This chapter describes the conceptual design and development of a microwave assisted process for extraction of tulsi essential oil. The materials used for fabrication of the various components and the instrumentation employed for measurement of parameters were explained. The process of evaluation of the system developed towards extraction of tulsi oil, and optimisation of the process parameters and characterisation of the microwave steam distilled tulsi oil in comparison with conventional steam distilled oil were detailed.

3.1 DEVELOPMENT OF MICROWAVE ASSISTED UNIT FOR EXTRACTION OF OIL

Based on a thorough review of works carried out on microwave assisted oil extraction, the design of a small capacity oil extraction unit assisted by microwave was conceptualized, further refined and then fabricated at K.C.A.E.T, Tavanur (Claudia, 2017). The developed experimental system as shown in Fig 3.1 and Plate 3.1 consists of the following main components:

1. Microwave reactor
2. Steam generator
3. Cartridge
4. Condenser

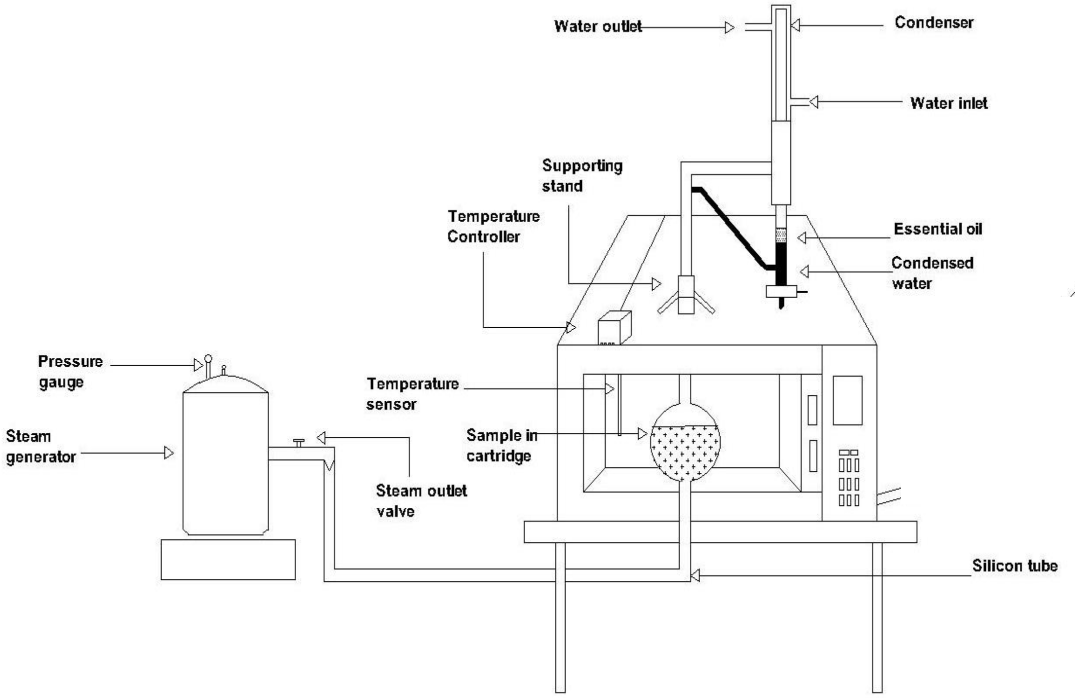

Fig 3.1 Schematic diagram of Microwave extraction unit

Plate 3.1: Microwave steam distillation

3.1.1 Microwave Reactor

Major requirement for the microwave steam distillation process for the extraction is a microwave reactor. Commercially available microwave ovens could be effectively utilised for this purpose (Plate 3.2). For laboratory scale experiments, ovens with power delivery of 700 W was chosen (Chemat *et al*., 2006; Sahraoui *et al*., 2011; Farhat *et al*., 2011; Kusuma *et al*., 2016).

The selected microwave oven has a control panel where time, power levels and action indicators are displayed. The microwave oven is equipped with five power levels such as 140 W, 280 W, 420 W, 560 W and 700 W for giving maximum flexibility and control over microwave application. The process of oil extraction was performed at power levels of 280 W, 420 W and 560 W. The time for extracting the oil can be set on the control panel.

Accordingly, a microwave oven (Model: LG MH2044DB) with following specifications was used to serve as the microwave source.

Table 3.1: Specifications of microwave oven

Power consumption	230 V/50 Hz, 700 W (Microwave Output)
	980 W (Grill Input)
	2000 W (Combination Input, Max)
Microwave frequency	2450 MHz
Outside dimensions	260 mm(h) x 455 mm(w) x 340 mm(D)
Oven cavity dimensions	185 mm(h) x 275 mm(w) x 250 mm(D)
Oven capacity	20 litres

3.1.2 Steam generator

The steam generator (10 L capacity pressure vessel) placed outside the microwave oven produced steam from water by using LPG as heating medium. Steam outlet from steam generator was connected to the cartridge containing tulsi via silicon tube (Plate 3.3.). The flow of steam to cartridge was controlled by employing a regulatory valve. Pressure of steam generated was measured using pressure gauge (Micro EN837-1; 0-3.5 kg cm-2 or 0-50 psi) (Plate 3.4.) attached to the lid of pressure vessel.

Plate 3.2: Microwave oven

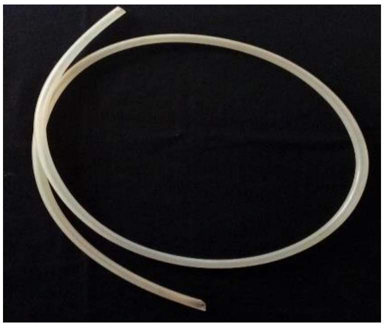

Plate 3.3: Silicon tube

Plate 3.4: Pressure gauge

Plate 3.5: Cartridge

3.1.3 Cartridge

The cartridge made up of microwavable glass. It’s size is in such a way that which fits with microwave oven cavity for the ease in loading and unloading of plant material. It has an outside diameter of 100 mm and volume of 80 ml. Cartridge has opening at top and bottom. Top neck has 25 mm length, with 32 mm outside diameter opening. The end of the top neck has conical ground glass joint (25 mm) which can accept any similarly-sized tapered fittings. Bottom stem has 65 mm length, with 11 mm outside diameter opening (Plate 3.5). Silicon tube carrying the steam from steam generator, was connected to the bottom stem of cartridge through a hole drilled on the bottom (13 mm diameter) of the microwave cavity.

3.1.4 Extraction unit

Extraction unit, placed outside the microwave oven, consists of clevenger apparatus and condenser. Clevenger apparatus which comprises of 10ml graduated receiver with stopcock, is a tool for essential oil extraction using steam. Condenser which act as a cooling system, condense the distillate continuously and thus separates the essential oil (Plate 3.6). A hole was drilled on the top (½ inch diameter) of the microwave cavity in order to fix the condenser into the cartridge which was positioned in the cavity. The cartridge and condenser was connected by means of a glass tube (12 mm x 100 mm) and two silicon rubber corks. A part of glass tube is projected upwards and remaining part is inside the oven cavity. The glass tube acts as carrier of both water and essential oil vapours.

Plate 3.6: Clevenger and condenser

3.2 EXPERIMENTAL DESIGN

The process parameters, power and time chosen as the independent variables that influence the essential oil yield and energy consumption which were taken as the dependent variables.

3.2.1 Independent variables

a. Microwave power (W)
    1. P_1: 280
    2. P_2: 420
    3. P_3: 560

b. Time of extraction (min)
    1. t_1: 25
    2. t_2: 35
    3. t_3: 45

3.2.2 Dependent variables

Microwave steam distillation system output parameters

a. Essential oil yield

3.3 EXPERIMENTAL PROCEDURE

Fresh tulsi leaves (*Ocimum tenuiflorum* syn *Ocimum sanctum*) (Plate 3.7) is collected from K.C.A.E.T Campus, Tavanur and used to evaluate microwave steam distillation process in the developed system. The detailed procedure for microwave steam distillation of tulsi essential oil is detailed below.

3.3.1 Extraction of Essential Oil

The steam generator used was filled half with water, closed and heated by LPG. 100 g of tulsi leaves is cut into small size and filled in the cartridge of the system (Plate 3.8). Cartridge was placed inside the microwave reactor one end of clevenger apparatus is connected to cartridge via glass tube and the other end to the condenser (Plate 3.9). The microwave power level and time of exposure was set in the control panel of microwave reactor for various treatment condition. Steam from the steam generator regulated by the steam control valve was allowed to pass through the cartridge via silicon tube. Microwave at present power level, heat the plant the materials for the set time interval. Essential oil in tulsi leaves gets vapourised and the passes out of the microwave cavity along with steam, through the distillation stem into the condenser and those vapors get condensed and fall into the stem of the clevenger apparatus where the oil and water get separated due to density difference. Oil sets collected as the top layer since it is lighter than water. After completion of the process the water was drained off by opening the valve and the oil was collected. Anhydrous sodium sulphate was added to essential oil for removal of moisture, and then stored in amber colored glass bottles at room temperature (Plate 3.10) for further analysis.

Plate 3.7 *Ocimum tenuiflorum*

Plate 3.8 Sample

Plate 3.9: Cartridge glass tube connection

Plate 3.10: Amber coloured glass bottle

The microwave assisted extraction is carried out in different power and time combinations as given in the section 3,2.1 and the best power and time is optimized by considering maximum yield of the essential oil. A comparison of physical quality characteristics, color, refractive index, solubility and specific gravity is carried out between oil extracted at optimum condition and oil extracted by hydro-distillation method.

The conventional hydro-distillation was carried out using Clevenger apparatus. It consists of a heating mantle and a round bottom flask filled with fresh cut tulsi leaves and water is added to round bottom flask at a ratio of 1:5. The one end Clevenger is fixed to round bottom flask and the other end with condenser for cooling the vapour mixture of essential oil and water.

3.4 DETERMINATION OF PHYSICAL QUALITY CHARACTERISTICS OF ESSENTIAL OIL

3.4.1 Specific Gravity

Specific gravity was determined by dividing the weight of one ml essential oil by the weight of one ml distilled water. Weights were calculated utilising a balance with an accuracy of 0.001g (Gopika and Ghuman, 2014).

3.4.2 Refractive index

The refractive index of tulsi essential oil was measured using Abbe refractometer (Advance Research Instrument company,Model : R8, India) Plate 3.11. A refractometer is used to determine a concentration of a particular substance within a given solution. It operates based on the principle of refraction. When rays of light pass from one medium in to another, they are bend either toward or away from a normal line between the two medium.

The sample is contained as a thin layer (approximately 0.1mm) between two prism. The upper prism is firmly mounted on a bearing that allows it rotation by means of the side arm shown in dotted lines.The lower prism is hinged to the upper to permits separation for cleaning and for introduction of the sample. The lower prism faces rough-ground: when light reflected in to the prism, this surface effectively becomes the source for an infinite number of rays that pass through the sample at all angles. The radiation is refracted at the interface of the sample and the smooth-ground face of the upper prism. After this it passes into the fixed telescope.

Two Amici prisms that can be rotated with respect to another serve to collect the divergent critical angle rays of different colours in to a single white beam, that correspond in path to that of the sodium D ray. The eyepiece of the telescope is provided with cross hairs: in making a measurement, the prism angle is changed until the light-dark interference just coincides with the cross hairs. The position of the prism is then established from the fixed scale.

The experimental step includes, firstly clean the surface of prism with alcohol using a cotton plug and put 2-3 drops of sample between the prisms using a dropper and press them together. Then allow the light to light to fall on the mirror and adjust the mirror to reflect maximum light in to prism box. Then rotate the prism box by moving lever until the boundary between shaded and bright parts appear in the field of view and if the band colour appear in the light shade boundary make it sharp by rotating the compensator. Then adjust the lever so that light shade boundary passes exactly the centre of the cross hair. Finally the refractive index can read directly from the scale.

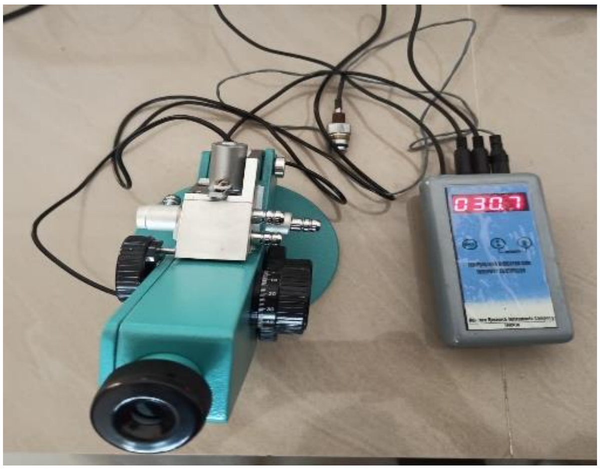

Plate 3.11 Abbe Refractometer

3.4.3 Solubility

The solubility of tulsi essential oil was determined based on the suggested by Food Chemical Codex (FCC, 1996). One ml sample of tulsi essential oil was transferred into a calibrated 10 ml glass stoppered cylinder graduated in 0.1 ml divisions. The oil was then diluted with 0.1 ml of 85 per cent (v/v) ethanol repeatedly each time. The temperature was maintained at 25° C, and the contents mixed thoroughly after each addition of alcohol. The dilution procedure was continued till a clear mixture observed. The volume of alcohol (V) utilised to obtain a completely clear solution was noted. Once the clear solution was obtained, the dilution process was continued, but with 0.5 ml 85 per cent ethanol till the volume of alcohol added was 20 times to the volume added earlier. The solution was shaken well each time with 0.5 ml ethanol until no turbidity was observed. The results were recorded as ‘‘one volume of essential oil soluble in V volumes or more of 85% ethanol”.

3.4.4 Color

The colour of the tulsi essential oil was found using a Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virgina, USA; model: HunterLab’s ColourFlex EZ) (Plate 3.12.).

Plate 3.12 Hunterlab colourflex meter

The Hunter lab’s colour flex spectro calorimeter consists of measurement (sample) port, opaque cover and display unit. This colour flex meter operates on the theory of focusing the light and measuring energy reflected from the sample across the entire visible spectrum. For matching a sequence of colour across the visible spectrum, primary lights are required and describes the colour by mathematical model called as Hunter model. It reads the colour of sample in respect of L*, a* and b*values where, luminance (L) forms the vertical axis, which denotes whiteness to darkness. Chromatic portion of the solids is designated by: redness a (+), greenness a (-), yellowness b (+), and blueness b (-). A transparent glass cup filled with sample was placed over the port of the instrument and an opaque cover which act as a light trap to exclude the interference of external light was placed over the cup. Before actual measurements colour was calibrated by fixing the definite colours like white and black tiles. After calibration, the sample was placed over the port and values of ‘L*’, ‘a*’ and ‘b*’ were recorded and repeated three times.

Results and discussion

CHAPTER 1V

RESULTS AND DISCUSSION

This chapter deals with the evaluation of a microwave steam distillation system developed for the extraction of tulsi essential oil. The outcomes of the various experiments conducted to optimize the process parameters were discussed in detail and the effect of microwave steam distillation and conventional hydro-distillation processes on the physical quality characteristics of essential oil were explained.

4.1 EVALUATION OF MICROWAVE STEAM DISTILLATION SYSTEM

The microwave steam distillation system composed of microwave reactor, steam generator, cartridge, extraction unit as shown in Figure 3.1

The microwave reactor selected a maximum power of 700 W. Steam outlet from steam generator was connected to the cartridge containing tulsi via silicon tube. Pressure of steam generated was measured using pressure gauge. The extraction unit which mainly comprises of Clevenger apparatus and condenser. The energy consumed for extracting the tulsi essential oil was measured theoretically. The oil extracted by microwave along with steam produced in the steam generator crosses the sample inside the cartridge of the reactor. The plant materials respond differently to the action of microwaves because the heating is through kinetic effects and is a volumetric process. Hence the process parameters leading to the efficient extraction need to be standardized.

4.2 STANDARDISATION OF THE PROCESS PARAMETERS OF MICROWAVE STEAM DISTILLATION SYSTEM

A series of experiments were conducted to optimize the process parameters for the extraction of tulsi essential oil. Three levels microwave power (280, 420 and 560 W) and time of extraction (25, 35, 45 min) were employed for the experiments as input variables. The experiments were conducted as discussed in the chapter III under section 3.3. The process parameters for extracting tulsi essential oil were standardized and the results were tabulated in Table 4.1.

Table: 4.1 Effect of process parameters on output characteristics of Microwave steam distillation system

SI. No	Sample	Essential oil yield (ml)
1.	P_1T_1	0.8
2.	P_1T_2	1.2
3.	P_1T_3	1.4
4.	P_2T_1	1.1
5.	P_2T_2	1.6
6.	P_2T_3	1.6
7.	P_3T_1	-
8.	P_3T_2	-
9.	P_3T_3	-
10	C	1.4

4.3 EFFECT OF PROCESS PARAMETERS ON OUTPUT CHARACTERISTICS OF MICROWAVE STEAM DISTILLATION SYSTEM

4.3.1 Essential oil yield

The essential oil yield of tulsi obtained in various combinations of experiments are shown in Table 4.1. The total yield of oil is 1.6 ml from 100g sample. The maximum oil yield was obtained for a microwave power of 420 W and extraction time of 35 min.

The microwave power have significant effect on the essential oil yield. When the power increased from 280W to 420W the essential oil yield increased. At low microwave power 280W the yield seems to be less, this is may be due the temperature must not be sufficient to break the oil bearing glands. With increase of power to 420W the essential oil yield increased but when power increased beyond 420 W no yield was observed. At 560W the water is jumbed through the Clevenger and a water-oil emulsion formed and it not able to recovered and at 700W the same effects observed and also the tulsi leaves burned. This might be due to increase in temperature. Increasing of microwave power level in oil palm mesocarp may result in physical damage such as burning and overheating which causes dark coloration of extracted oil (Norashikin *et al*., 2021).

The essential oil yield also increased with increase in time of extraction. At 280 W power, when the time of extraction increased from 25 min to 35 min the oil yield is increased by 50 % and when time increased to 45 min the essential oil yield increased from 1.2 to 1.4 ml. At 420 W power, when time increased from 25 min to 35 min, the yield increased from 1.1 ml to 1.6 ml and when the time increased to 45 min there was no increase in essential oil yield. So it is concluded that maximum oil yield is found to be 1.6 ml from 100g of fresh leaves. Jorge A.Pino , 1998 reported that air dried aerial parts of *O. tenuiflorum* including stalks and leaves, were subjected to hydrodistillation in a Clevenger-type apparatus, with an oil yield of 1.6%.

In the conventional extraction process, the essential oil yield was 1.4 ml from 100g of leaves and the time taken was 5 h and when time increased to 6 h there have no change in the essential oil yield. So it is concluded when compared to MASD the yield is low also the time taken for extraction was high.

4.4 DETERMINATION OF PHYSICAL QUALITY CHARACTERISTICS OF THE TULSI ESSENTIAL OIL

The physical quality characteristics of hydrodistilled oil and the oil extracted using MASD at optimum process parameters were tabulated in Table 4.2

Table 4.2: Physical quality characteristics of tulsi essential oil

S/No	Sample	Specific gravity	Solubility (v/v)	Refractive index	L*	a*	b*
1	P_2T_2	0.9	1.5	1.517	22.8	-0.17	0.28
2	C	0.88	1.4	1.462	20.6	-0.2	0.24

4.4.1 Specific gravity

The specific gravity of tulsi essential oil obtained in both experiments were tabulated in Table 4.2. The values of specific gravity for MASD sample is 0.9 and conventionally extracted is 0.88. From the above values it is clear that there have a slight change in specific gravity of MASD oil and conventionally extracted oil.

The specific density of cumin essential oil extracted by traditional water distillation and microwave assisted water distillation reached 0.724 and 0.744, respectively (Sainin *et al*., 2014). The specific gravity of extracted Eucalyptus oil by water distillation method was 0.9162 and oil extracted by ohmic heating is 0.933, Therefore, the extracted essential oils from eucalyptus leaves by using ohmic heating is an extraction technology that extracts the essential oils with high quality (Abdull, 2009). So from the above two reviews it is concluded that specific gravity is a qualitative test for the purity of oils, so the MASD produces high quality oil or there have no difference in the quality of conventionally extracted and MASD extracted oil.

4.4.2 Solubility

The solubility of essential oil obtained in various experiments in 85% ethanol were tabulated in Table 4.2. The values of solubility varied between 1.7 and 1.4 v/v. The solubility values obtained in MASD tulsi essential oil were in close relation with conventional tulsi essential oil.

4.4.3 Refractive index

The value of refractive index of two samples were given in the Table 4.2. The refractive index of MASD oil is 1.517 and conventionally distilled oil is 1.462. There have a little change in the refractive indices of oil extracted using two techniques. The MASD oil have higher refractive index. Some studies showed that the refractive index is used to identify compounds, to determine their purity, and to analyze the ratio of homogeneous binary mixtures of known components. The latter occurs because the RI of a mixed solution is a weighted average of the RIs of the components of the mixture. Thus, it is useful as a rapid measure of purity and quality. Johannes. (2016) showed that the thymol content in oregano is decreased with decrease in the refractive index.

So, from the above reviews it is inferred that the MASD oil have more quality than the conventional oil and the refractive index is less for the conventional. It may be due the leaves are subjected to high temperature for about 5 hr but in MASD the process last at 35 min.

4.4.4 Colour

The colour of tulsi essential oil is determined by using Hunter lab colour flex meter and the values of L*, a* and b* obtained for various samples are given in Table 4.2. The L*, a*, and b* value of MASD oil was 22.8, -0.17 and 0.28 respectively. The L*, a*, and b* value of conventionally distilled oil was 20.6, -0.2 and 0.24 respectively. There have no significant change in the colour values of MASD extracted oil and conventionally extracted oil.

The increment in microwave power, the colour of oil changes to dark yellow or even black because of the presence of suspended materials (Baron and Villa, 2014). But in this case the change in L*,a*,b* values is very less so it is inferred that the oil is obtained by MSD process is clear.

Summary and conclusion

CHAPTER V
SUMMARY AND CONCLUSION

The essential oil are aromatic oily liquids extracted from different parts of aromatic plants. These volatile oils represent the typical flavour and aroma of a particular plant from which they are obtained. They are low-volume, very high value products and contain a complex mix of components. These oils are used principally in perfumery and food flavourings.

There are various various techniques for essential oils such as hydro distillation and steam distillation on the basis of distinct principles of difference in vapour pressure. The major shortcomings of the conventional methods are low yields, long extraction time, high energy consumption, high cost, thermal and hydrolytic degradation and environmental pollution.

Nowadays there have many emerging novel technologies, like microwave, ultrasonic etc which enhances the extraction technique by decreasing time of extraction and energy consumption same time the essential yield is improved. Such as innovative technology microwave assisted steam distillation. In this process microwave radiation is only applied on the extraction reactor, which is the basis of steam distillation principle. In microwave steam distillation process, while the plant material is subjected to microwave radiation, steam generated outside permeate through the plant material, evaporates and carries the essential oil towards the condenser where it is separated and collected. The microwave penetrates through the oil-bearing glands and the extraction efficiency is increased.

The developed microwave steam distillation system composed of a microwave reactor, steam generator, cartridge, extraction unit, Clevenger apparatus, heating mantle and temperature sensor.

In order to evaluate the developed system towards extraction of tulsi essential oil, the process parameters which would influence the essential oil yield and were chosen as independent variables after preliminary studies. The microwave power and time of extraction is taken as the independent variables. A series of experiment is carried out with different temperature and time combination to optimize the process parameters. And the essential oil was extracted by setting the optimized parameters. The hydro distillation of tulsi essential was also conducted for a comparison with MASD, where the hydro distillation is a conventional method.

The optimized process parameters corresponding to maximum yield are 420W and 35 Min. The oil extracted by MASD using the power 420W for 35 min was compared with conventionally distilled oil in terms of specific gravity, Refractive index, colour and solubility.

The specific gravity of MASD oil is 0.9 and hydro distilled id 0.88 and the solubility of MASD oil is 1.5 and hydrodistilled is 1.4. The refractive index of MASD and hydro distilled is 1.517 and 1.462 respectively, a significant change in values is observed in this case. The L*, a*, and b* value of MASD oil was 22.8, -0.17 and 0.28 respectively. The L*, a*, and b* value of conventionally distilled oil was 20.6, -0.2 and 0.24 respectively.

There is no significant change in physical quality characteristics, except the refractive index. The refractive index of microwave assisted steam distilled oil is high when compared with conventionally distilled oil, indicates it’s superior quality. The quality of essential oil mainly evaluated by determining its chemical constituents, degradation in any of the chemical constituents decreases its quality there by the refractive index also. To determine the constituents quantitatively and also to prove the superior quality of the oil several chromatographic techniques can be used.

References

CHAPTER VI

REFERENCES

Anonymous. 1999. Council of European Committee of Experts on Flavouring Substances, Publication Datasheet on Methyl Eugenol, Document RD 4.14/2-45

Anonymous. 2001. Opinion of the Scientific Committee on Food on Methyl Eugenol (4-Allyl-1,2-Dimethoxybenzene), Health and Consumer Protection Directorate General, European Commission, Brussels, Belgium.

Balaji, R., Prakash, G., Suganya, D.P, Aravinthan K, M. 2011 Antioxidant activity of methanol extract of *Ocimum tenuiflorum* (dried leaf and stem), IJPRD 3(1):20-27.

B.B. Dey, M.A. Choudhuri, Biochem. Physiol. Pflanz. 178 1983 331.

Boldor, D., Sanders, T.H. & Simunovic, J. 2004. Dielectric properties of in-shell and shelled peanuts at microwave frequencies. Transactions of the ASAE, 47: 1159-1169.

Boldor, D., Sanders, T.H., Swartzel, K.R. & Farkas, B.E. 2005. A model for temperature and moisture distribution during continuous microwave drying. *J. of Food Process Engg*. 28: 68-87.

Bousbia, N., Vian, M., Farhat, M., Meklati, B. andChemat, F. 2009. A new process forextraction of essential oil from citrus peels: Microwave hydro-diffusion and gravity. *J. Food Engg*. 90: 409-413.

Chandrasekaran, S., Ramanathan, S. and Tanmay, B. 2013. Microwave food processing – a review. *Food Res. Int*. 52: 243-261.

Chemat, F., Vian, M.A. and Fernandez, X. 2013. Microwave-Assisted Extraction of Essential Oils and Aromas. In: Cravotto,G., and Chemat, F. (eds), Microwave assisted extraction for bioactive compounds Theory and Practice. Springer Science+Business Media, New York. 53-68p.

Claudia, K. L. 2018. Studies on microwave steam distillation process for extraction of lemon grass essential oil. MTech. (Ag) thesis, Kerala Agricultural University, Thrissur, 84p.

Coronel, P., Truong, VD., Simunovic, J., Sandeep, K.P. and Cartwright, G.D. 2005. Aseptic processing of sweet potato purees using a continuous flow microwave system. *J. of Food Sci.* 70: 531-536.

Datta, A.K. and Anantheswaran, R.C. 2000. Handbook of microwave technology for food applications. Marcel Dekker Inc, New York, 558p.

Gajula, D., Boateng, V. J., Shackelford, M. S. R., Sims, C., Asiamah, D. and Walker, L.T. 2010. Basil reduces azoxymethane induced colon tumours in fisher 344 male rats. Res. *J. Phytochem*.1-10p.

Ghule, R. S., Venkatnarayanan, R., Zope, V. N. and Upadhyay, U. M.2010. Larvicidal activity of essential oil obtained from different species of genus Ocimum against mosquito vector. *Deccan J Natural Products*. 1(2):6-8.

Guenther, E. 1984. The Constituents of Essential Oils, The Essential Oils, Robert E. Krieger Publishing Company, Malabar, FL, USA. 2: 519–520.

Guo, W., Liu, Y., Zhu, X. and Wang, S. 2011. Temperature-dependent dielectric properties of honey associated with dielectric heating. *J. Food Engg.* 102: 209-216.

Icier, F. and Baysal, T. 2004b. Dielectrical properties of food materials – 2: Measurement techniques. *Crit. Rev Food Sci. Nutr*. 44:473-478.

Icoz, D., Sumnu, G. and Sahin, S. 2004. Color and texture development during microwave and conventional baking of breads. *Int. J. Food Properties*. 7: 201-213.

Johri, PK., Tiwari, D. and Johri,R. 2009. Screening of some indigenous medicinal plants for antiimplantation / anti- fertility activity in female albino rats. *Biochem Cell*. 9(2):175-178.

Joshi and Parle. 2006. Cholinergic basis of memory improving activity of *Ocimum tenuiflorum* Linn. Int. J. Pharmaceutical Sci. 68(3):364-365.

Kusuma, H.S., Putra, A.F.P. and Mahfud, M. 2016. Comparison of two isolation methods for essential oils from orange peel (*Citrus auranticum* L) as a growth promoter for fish: microwave steam distillation and conventional steam distillation. *J. Aquac. Res*. Dev. 7(2):1-5.

Laakso, L., Seppanen-Laakso, T., Hermann-Wolf, B. K. Knobloch. 1990. Constituents of the essential oil from the Holy Basil or Tulsiplant, *Ocimum sanctum*. *Planta Med*.56: 527.

Lau, M.H. and Tang, J. 2002. Pasteurization of pickled asparagus using 915 MHz microwaves. *J. Food Engg*. 51: 283-290.

Lewis, M. E. 2001. Should we be concerned about herbal remedies?. *J.Ethnopharmacol*. 75:141-164.

Lawrence,B.M.,Hogg,J.W.,Terhune, S.J. and Pichitakul, N. 1972. Flavor Ind. 3:47.

Lawrence,B.M.,Powell,R.H.,Peele,D.M. 1980. in: Proceedings of the VIIIth International Congress of Essential Oils, Cannes, Fedarom Grasse. 111–117p.

Machado, M.I.L.,Silva, M.G.V.,Matos,F.J.A. Craveiro, A.A.,andAlencar,J.W. J.1999. *Essent. Oil Res*. 11: 324.

Maheshwari,M.L.,Singh,B.M.,Gupta,R.,Chien,M. 1987.Essential oil of sacred Basil. *Indian perfumer*.31:137-145.

Malik,M.S., Rafique, M. and Ahmed, R. 1986.Pakistan *J. Sci. Ind. Res*. 29: 111.

Metaxas, A.C., and Meredith, R.J. 1983. Industrial Microwave Heating. London: Peter Peregrinus Ltd.

Morales, M.E. 2012. Inactivation of Salmonella Typhimurium in fresh vegetables using water-assisted microwave heating. *Food Control*.26:19-22.

Mohammed, T. G. and Rezaei, K. 2008. Comparison of microwave-assisted hydro distillation with the traditional hydro-distillation method in the extraction of essential oil from *Thymus vulgaris* L. *Food Chem*. 109: 925-930.

Pino, J.A.,Rosado,A., Fuentes, V.(1996).Composition of the essential oil from the leaves and flowers of *Ocimum gratissimum* L. grown in Cuba. *J. Essent. oil Res.*139-141.

Poucher,W.A.&JouharA. J. 1991. The Raw Materials of Perfumery, Poucher's Perfumes, Cosmetics and Soaps, vol. 1, ninth ed., Chapman& Hall, London, UK. 148–149p.

Praveen, K. P, Prabhakara, M. C., Satyavathi,K. and Anil K. S.2010. Evaluation of cardiac activity of some traditionally used backyard Indian medicinal plants. *Res.J. Pharm, Biol and Chem* Sci.1(4):641-654.

Pushpangadan,P.,Bradu, B.L.,Chaddha,K.L. and Gupta R. 1995. Medicinal and Aromatic Plants: Advances in Horticulture, vol. 11,Malhotra Publishing House, New Delhi, India. 628–657p.

Racotia,A.,Buttressb, A. J.,Binnerb, E.,Doddsb, C.,Trifana, A. and Ioan, C. 2017. Microwave assisted hydro-distillation of essential oils from fresh ginger root. *Zingiber officinale Roscoe*.471-480p.

Ravi, P., Elumalai, A.,Eswaraiah, M. C. and Kasarla, R. 2012. A review on Krishna tulsi, *Ocimum tenuiflorum* linn. *Int.J.Res. Ayurveda and Pharm*. 3(2):291-293.

Ryynanen, S. 1995. The electromagnetic properties of food materials: a review of the basic principles. *J. Food Engg*.26: 409-429.

Sermakkani, M. and Thangapandian, V. 2011. Studies on preliminary phytochemical constituents and antimicrobial activity of *Ocimum tenuiflorum* L leaves. *Int. J. Institutional Pharm Life Sci*.; 1(1):1-12.

Sharma, A., Meena, A and Meena, R. K. 2011. Biochemical estimation of primary metabolites. *Ocimum tenuiflorum*. 6(3):463-465.

Sharma, G.P. and Prasad, S. 2001. Drying of garlic (Allium sativum) cloves by microwave-hot air combination. *J. Food Engg*, 50: 99-105.

Simon, J. E, Quinn, J. and Murray, R. G. 1999. Basil: a source of essential oils. In: Janick J, Simon J E. (Eds). Advanced in New Crops. Timber Press, Portland :484-489.

Singh, M., Raghavan, B. andAbraham, K.O. 1996. Processing of marjoram (Marjona hortensis Moench.) and rosemary (Rosmarinus officinalis L.). Effect of blanching methods on quality. 1996. *Food/Nahrung*.40: 264-266

Soysal, Y. 2004. Microwave drying characteristics of parsley. *Biosystems Engg*., 89:167-173.

Sudha, P., Zinjarde, S. S., Bhargava, S. Y. and Ameeta, R. K. 2011. Potent a-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement and Altern Med* 11(5):1-10.

TNAU (Tamilnadu Agricultural University).2021.Annual Report 2020-2021.Tamilnadu Agricilural Univesity.

Trevisan, M. T., Vasconcelos S. M. G., Pfundstein, B., Spiegelhalder, B. and Owen, R. W. 2000. Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus *Ocimum*. *J. Agric. Food. Chem.*

Vimalan,A.K.,Sadanandan,K.,Philip,M.P.,Natrajan,N., Murugesan, M. and Damodaran,N.P. (1989). Proceedings of the 11th International Congress of

Essential Oils, Fragrances and Flavours, New Delhi, India. 181–188p.Warrier, P. K. 1995. Indian Medicinal Plants. Orient Longman. 168p.

Yonei, Y.,Ohinata,H.,Yoshida, R., Shimizu, Y. and Yokoyama, C. 1995. Extraction of ginger flavor with liquid or supercritical carbon dioxide. *J. Supercrit Fluid*. 8(2):156–161.

Zhang, M., Tang, J., Mujumdar, A.S. and Wang, S. 2006. Trends in microwave related drying of fruits and vegetables. *Trends Food Sci. Technol*. 17: 524-534.

STUDIES ON MICROWAVE STEAM DISTILLATION PROCESS FOR EXTRACTION OF TULSI ESSENTIAL OIL

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ABSTRACT OF PROJECT REPORT

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ABTRACT

Essential oils are concentrated aromatic oily liquids distilled from different parts of aromatic plants. Conventionally steam distillation has been widely used for extraction but it have some disadvantages. Modern technologies have been continuously developed to overcome the disadvantages of conventional methods. Microwave steam distillation is based on the interaction between water in the plant material and microwaves generated by the energy source. In this process, the steam generated outside accelerates evaporating and carrying of the essential oil, from the plant material, towards the condenser. In this study microwave steam distillation was used for extracting tulsi essential oil in a system that was already developed in the KCAET campus which is composed of microwave reactor, steam generator, cartridge, extraction unit, supporting stand, energy meter and temperature sensor and controller. In order to evaluate the developed system towards extraction of tulsi essential oil, the effect of process parameters which would influence the essential oil yield, microwave powers of 280, 420 and 560 W were studied. The physical quality characteristics such as specific gravity, refractive index, solubility and colour of essential oil were analysed. The optimised operating microwave power and time of extraction for tulsi essential oil in microwave steam distillation were found to be of 420 W and 35 min respectively. From the study it was concluded that microwave steam distillation could be considered as an extraction technique that results in the rapid production of high quality essential oil at shorter extraction period with minimum energy consumption.