

A
Dissertation Report
on

**QUANTATIVE AND QUALITATIVE ANALYSIS OF
ESSENTIAL OIL OBTAINED FROM TURMERIC RHIZOMES**

Submitted in partial fulfillment of the requirement for the award of the degree of

**MASTER OF SCIENCE
IN
CHEMISTRY**

Submitted by
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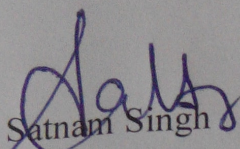
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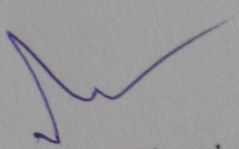
Certificate

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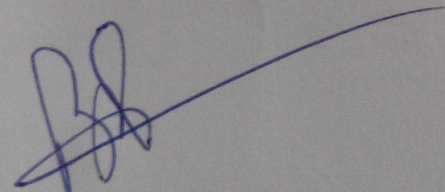
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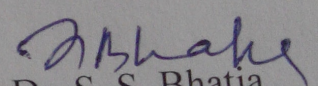


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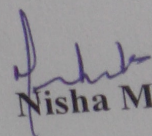
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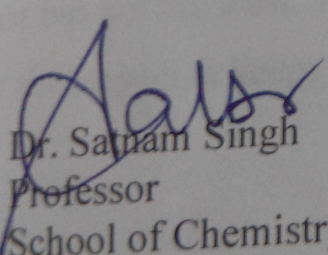
I hereby declare that the work being presented in the dissertation entitled "**Quantitative and qualitative analysis of essential of obtained from Turmeric Rhizomes**" in partial fulfillment of the requirements for the award of degree of Master of Science in Chemistry, and being submitted to School of Chemistry & Biochemistry, Thapar University, Patiala, is my own work during the period of January to July 2016, under the supervision of **Dr. Satnam Singh (school of chemistry and biochemistry)** and **Dr. Avinash Chandra (department of chemical engineering)**. I have not submitted the contents embodied in this dissertation for the award of any other degree.

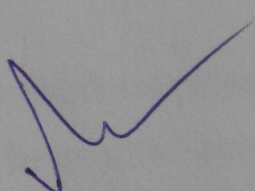
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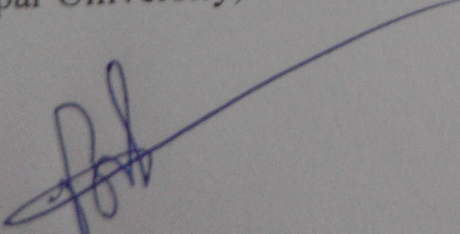
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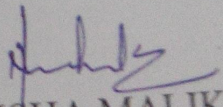
My years of Post graduate study are finally nearing completion. This is the opportune moment to acknowledge all who have crossed my path during the journey and contributed directly or indirectly to reaching the light at the end of the tunnel.

Undoubtedly, my supervisors, **Dr. AVINASH CHANDRA** (*Department of Chemical Engineering*) and **Dr. SATNAM SINGH** (*School of Chemistry and Biochemistry*) deserve to be at the top of the list. It was an excellent opportunity to work under the tutelage of two stalwarts of Chemical Engineering and Chemistry department. These years have been helpful for my academic progress. I sincerely thank them for their kind guidance, helpful suggestions, encouragement. The fruitful discussions with them throughout the research work were a great help for the successful completion of the present work.

It is a great pleasure to thank the authorities of Thapar University, Patiala for providing the opportunity to carry out my research work successfully.

I owe sincere and earnest thankfulness to my family who enthused, encouraged and fully supported me for every trial comes in my life. I acknowledge my sincere thanks to all my friends for providing the companionship and making my stay at Thapar University, Patiala pleasant and joyful.

Last but not the least, To God the father of all, I thank for the strength that keeps me standing and for the hope that keeps me believing that this affiliation would be possible and more interesting.


NISHA MALIK

ABSTRACT

Turmeric is common spice, widely used in Indian kitchen as food additive and preservatives. It is also used as effective medicines in Ayurveda. The essential oil is isolated from fresh turmeric rhizomes using modified steam distillation process. The obtained oil yield is compared with the oil obtained from conventional process. The oil yield from fresh rhizomes is obtained as~2.83-2.48%. The obtained yield is 13% more in rhizomes as compare to conventional processes. It is found that the loss of water soluble polar compound is less as compared to conventional process.

The main constituents of turmeric essential oil are

ar-turmerone, Curlone, Zingiberene, ar-curcumin, Dehydro curcumene and ~60% compound were not identified. Overall, the modified process is feasible to obtain the essential oil from the turmeric rhizomes with increase yield and reduced loss of polar compounds

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Chapter-1

INTRODUCTION

India has a record history of foreign trade of spices and herbs since ancient and times. The spices production and marketing are the economic stay of millions of people of our country. India is reputed as the “Land Of Spices” and around 65 spices/ herbs crops are being grown in our country at present.

American spices trade association (ASTA) defines spice as “Any dried plant product used primarily for seasoning purpose.” The spices contribute aroma, taste, flavor, colour and pungency to food rather than an alone food seasoning factor. Spices may bark buds flowers, fruits, leaves roots seeds or the entire plant. They are well known as preservatives and many of them have rich medicinal properties and are used in pharmaceutical, perfumery, cosmetics products, and religious rituals etc.

India is the largest producer of spices and they are grown all over the country like Saffron in Kashmir; celery in Punjab; hot chilies and large cardamom in north eastern regions; cumin, coriander and dill in Rajasthan and Gujarat; Pepper, cardamom, ginger, turmeric and chilies in the Southern States.

Spices in their raw forms have certain disadvantages. Ground spices do not impart their total flavor. The finally ground spices; lose their total flavor during grinding process. The species lose their precious aromatic constituents (fully and/ or partially) due to their volatile nature. It is bulky for storage and often unhygienic due to the bacterial contamination. The extraction of essential oil and production of oleoresins from spices/ herbs is one way to store the spices for long time without losing any aromatic constituents.

The steam distillation is the common technique to extract essential oil from plant materials. Then the extracted essential oil is converted in to oleoresins using organic solvents. The oleoresin

contains all the active flavoring principal ingredients of the spice, containing both volatile oil and non-volatile resin. Usually, the essential oil is the mixture of large number of components.

The essential oil constituents/ components are also derived from turpentine and petroleum sources for their commercial use.

These materials are processed on very large scale so economy of scale favors final prices of this synthetic derived molecule at present compare to equivalent natural isolates. However we should take in consideration following factor for future which can favor production of these isolates, derived from natural essential oils.

1. Prices of petroleum products are going up, so in future with depletion of it prices are expected to rise further.
2. Recovery of turpentine oil from crude sulfate turpentine liquor requires a lot of energy which is derived from petroleum as fuel so it leads additional hike in production cost. It is expected that same trend is going to continue in future. Again for separation and conversion of alpha-pinene and beta-pinene from turpentine to its desired basic derivative like Geraniol, Citronellal, Citral also requires lot of energy and chemicals, which are derived from petroleum feed stock.
3. Conversion of petroleum feed stock and turpentine to above mentioned derivatives require use of lot of hazardous reactions, which leads to generation of many pollutants as well hazards. Now with more emphasis world over on neat and clean technology these facilities of production derivative like curcumin, Geraniol, Citral, at very low cost as they may have to spend lot of money on pollution control and safety of operation.

Overall, the production of essential oil from the plant origin is cheaper, environment friendly and green.

1.1 *Curcuma Longa L.* (Turmeric)



Figure 1: Rhizomes of turmeric

The botanical name of turmeric is *Curcuma Longa L.* and it belongs to zingiberaceae family. is highly. The golden-yellow color of turmeric is resembles the sunlight, reported in the Vedic literature, so it is highly admired by the Indo-European people, for its golden-yellow color

Turmeric, best known as “Harida” in Sanskrit, has rich history in India and has been used for centuries in the Ayurvedic system of medicine. It can be identified by forty six synonyms such as Pita “yellow”, gauri “brilliant”, etc.

The yellow colour of turmeric, which is resistant to very high dilutions, found its way to commercial use as a colouring agent for various items including cotton, silk paper, wood food stuffs and cosmetics.

Turmeric became of special importance to man with discovery to that its powdered rhizomes when added to various food preparations preserved their freshness and nutritive value. Turmeric which belonged to a group of aromatic spices was originally used as a food additive in curries to improve storage conditions, palatability and preservation of food. Long before the time of cheaper synthetic food preservative, spices like turmeric played a vital role as food additives and were valued more than gold and precious stones.

In Ayurveda, turmeric has been used internally as a stomachic, tonic and blood purifier and topically in the prevention and treatment of skin diseases. It has been prescribed in liver diseases, and particularly for jaundice, and urinary tract diseases. In chronic catarrh and coryza, the inhalation of the fumes of burning turmeric causes copious mucous discharge and gives instant relief.

In the present work, the essential oil was extracted from turmeric fresh rhizomes using modified steam distillation process. The quantitative and qualitative analysis is performed of obtained essential oil from turmeric.

Turmeric has anti-inflammatory, nematocidal activities anti-human immunodeficiency virus. *Curcuma longa* L. handle all the biological actions while curcumin is responsible for anti-inflammatory effects. It also stops the cancer growth and also carcinogenesis.

Chapter 2

LITERATURE SURVEY

The chemical composition of oil isolated from the leaves of *Curcuma longa* (turmeric) was studied by *Prathapan et al. (2012)*. It was analyzed using GC-MS which showed that sequephellandrene (22.8%) followed by terpinolene (9.5%) are the major compound present in the leaf oil of the turmeric. Turmeric oil is capable of retarding oxidation reaction and is free radical mediated damage.

Antimicrobial activity of volatile oil in dried powder were investigated by *Singh et al. (2011)* by hydro-distillation and total curcuminoids content was evaluated by UV spectrophotometer. In dried turmeric powder, average yield of volatile oil is 8.20 ± 1.66 %v/w and total curminoids is 7.57 ± 0.04 % w/w. For antibacterial activity they were evaluated against medically important bacteria by Agar diffusion method and it showed better results as compared to volatile extracted from turmeric.

Hydro-distillation was done for the essential oil of the rhizomes harvested from 27 different accession of *curcuma longa* grown in Lucknow by *Garg et al. (1999)* On the basis of major terpenes the essential oil was evaluated. It varies from 0.61% to 1.45% when evaluated for curcumin content on dry basis.

Solvent extraction method is used by *Jain et al. (2007)* for isolating herbal medicament from rhizomes of *curcuma longa*. GC-MS and HPLC was done for identification and standardization of herbal medicaments. 0.32-0.55% bioactive constituents and 50-90% Curcuminoids was found in herbal medicament. It was also investigated that herbal medicaments are light sensitive and are stable at different temperature and pH.

Chemical composition of tissues of *curcuma longa* L. was studied by *Li et al. (2011)*. 235 compounds were discovered by using chromatographic and NMR techniques. It was found that the product quality of turmeric oil is controlled by curcumin.

The major components of oil were analysed by GC-MS of essential oil by *Ferreira et al. (2013)* *Aspergillus flavus* was used to study the antifungal activities of oil for growth inhibition. It was found that *curcuma longa* essential oil reduces the growth rate of *Aspergillus flavus* at 0.10%. Hyphae membranes and conidiophores of *Aspergillus flavus* is damaged when exposed to oil showed by Scanning electron Microscopy (SEM).

Fresh and dried rhizomes upon antioxidant potential grown in vitro from four clones of turmeric is studied by *Cousins et al. (2007)*. From iron chelation it is noted that recently dried tissue are more useful than the commercial one.

A new process design and operation for steam distillation of essential oils that increases oil yield and reduces loss of polar components in waste water was developed by *Masango (2004)*.

A packed bed of raw material was used the method also addresses one of the most difficult problem in steam distillation, that of determining the optimum amount of steam required for distillation of raw material. Steam distillation process result in improved recovery of valuable essential oils, energy saving and environmental improvements. The new packed column design reduced oil losses considerably when compared with other distillation configurations. The odour nuisance is eliminated or reduced.

The variation of biochemical contents in leaves petioles and stem of *Curcuma longa* L. reported *Niranjan et al. (2003)* different sample of turmeric from Agra, Allahabad, Dehradun,

Kanpur, Lucknow, Mirzapur, Sultanpur and Varanasi. The volatile oil content varies 2.1%-3.4% in rhizomes.

On line High Performance Liquid Chromatography-UV Diode Array and Electrospray Mass Spectrometry of turmeric showed the presence of the five major compounds. Identification based on their spectra, mass spectra, and retention times in comparison with the data of standard compounds. *He et al. (1998)* suggested this method provides a reliable finger prints for turmeric distinguishing it from other species.

Eight synthetic curcuminoids were investigated by *Anto et al. (1996)* their cytotoxic and tumoricidal activities as well as their free radical scavenging activity. As antitumor agents veratryl curcuminoids and salicyl curcuminoid increase life span of animals and human by 100.6% and 86.9% respectively.

Pfeiffer et al. (2003) studied the stability of turmeric constituents, the curcuminoids decomposes vary rapidly when serum was omitted but were more stable in the presence of serum. The stability differed between the curcuminoids. It has been observed that all the curcuminoids are unstable.

Tomaino et al. (2005) studied the effect of heating on antioxidant effectiveness and the chemical composition of same essential oil. The essential oils under study exhibited good antioxidant properties and might be effectively used to control lipid oxidation during food processing.

Major pigment from turmeric shows a variety of pharmacological properties such as anticancer, antioxidant and anti-inflammatory. It also exhibits photo biological activity Absorption and Fluorescence Spectra of curcumin and its methoxy derivative were recorded by *Barik et al. (2002)*.

Reverchon (1997) reported a comprehensive overview of the analytical, processing and modeling aspects. That was partly due to the difficulties involved in isolating essential oils from the other products, which supercritical CO₂ can dissolve. Process proposed to isolate and fractionate essential oil by supercritical CO₂ and the responding modelling aspects are discussed critically.

The leaf oil of two cultivars of turmeric was investigated by GC. The leaf oil characterized by **Behura et al. (2004)**, Myrcene, Terpinolene, Cymene were the major constituents. The volatile oil obtained from the tuber of *Curcuma longa L.* is an orange-yellow, occasionally slightly fluorescent liquid, with an odour reminiscent to the tubes

Gunther et al. (1984) reported physiochemical properties of turmeric oil i.e. specific gravity (0.938-0.967), refractive index (1.512-1.517), solubility etc.

Eleven samples of *Curcuma longa L.* from South-Indian were studied. The two monoterpenes α -pinene and β -pinene were identified by **Visan et al. (1986)**. For the first time in essential oil. Thin Layer Chromatography (TLC) and Gas Chromatography (GC) also determined the curcumin contents.

The essential oil yield from the leaves of *Curcuma longa L.* was 2.8% on dry weight basis the essential was evaluated for its major terpenes **Garg et al. (2002)**.

Plants of *Curcuma longa L.* were grown in agro climatic conditions of north Indian planes. Hydro-distillation of leaves and rhizomes each 2.2% oil yield, which were analyzed by GC and GC-MS.

Rhizome and leaves contained 84,83 compounds respectively. The experiment was carried out by

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Raina et al. (2001)

Overall, the proposed work is the gap in the available body of literature.

Chapter 3

METHOD AND MATERIAL

3.1 Conventional methods of Distillation

Distillation is one of the most important production methods for Essential oils. Its basic principle depends on the botanical material and also on the conditions of the material. There are many methods used for distillation of essential oil like:

- 1) Water Distillation
- 2) Water and Steam Distillation
- 3) Direct Distillation

Water Distillation is used for the plants which has been dried and not be damaged by boiling. It is also used the powdered materials that need to float freely as they tend to lump together when just steam is passed through them. The mixture is heated until the plant and oil are condensed in the condensing chamber.

Water and Steam Distillation is used for either fresh or dried plant material that would be damaged by boiling. The plant material is supported on a perforated grid. The water level below the grid and low pressure, wet steam passes through the plant material. The most important aspect of this method is that the steam is never really hot and always at low pressure. Cinnamon and clove oils are prepared by this method.

Direct Distillation is passed through plant material which can be anything from wood to flowers, and which has been pressed tightly and evenly in a still. The heat of the steam bursts opens the membrane of the oil receptacles in the plant and the volatile plant oil molecules are released into the steam. The steam rises and takes with it hydrophobic, and hydrophilic molecules. The steam rises and takes with it hydrophobic, and hydrophilic molecules. This mixture of water and various other molecules of the plant components separate back into liquid by means of being condensed.

The distillation process itself is carefully undertaken, not only with regards to the materials used but also that the temperature is neither too high nor too low and timing is neither too short nor too long.

3.2 Present Experimental Process:

The modified process has been adopted in the present study to increase the essential oil yield and reduce the loss of water soluble polar compound. In the present modified steam distillation process, the water is being circulated and reused in the boiler. The experimental setup is made up of glass. All the process components are made of borocil glass and assembled in a sequence shown in Fig. 1

3.3 Feed stock:

The fresh turmeric was collected from the markets of Patiala and Chandigarh. It has been ensured that the cultivation of turmeric was done in north Indian region. The fresh turmeric was washed and shed dried to remove the dirt and mud. The turmeric were chopped in small slices to prepare feedstock. This chopped slice turmeric were fed to the packed column of the experimental setup.

3.4 Experimental set-up and procedure:

The steam is continuously produced in flask A through electrical heating and passes through turmeric column containing random packing of longitudinal slices of turmeric rhizomes in the column. The empty top portion of the column was filled with glass wool. The vapours rising from the column mainly comprising of water vapours and lighter components of turmeric oil were passed through a condenser D, where the water vapours and condensed components were taken into the collector E. The oil being lighter remained in the collector as top, whereas condensed water layer remained as bottom layer. In the continuous operation loss of water by way of boiling

was compensated continuously from condensed water from column F. Finally, after 6 hours, operation, heating was stopped and the product (essential oil) was drained through column F from a tap G. Vent H was provided for periodically release of uncondensed gases and excessive pressure

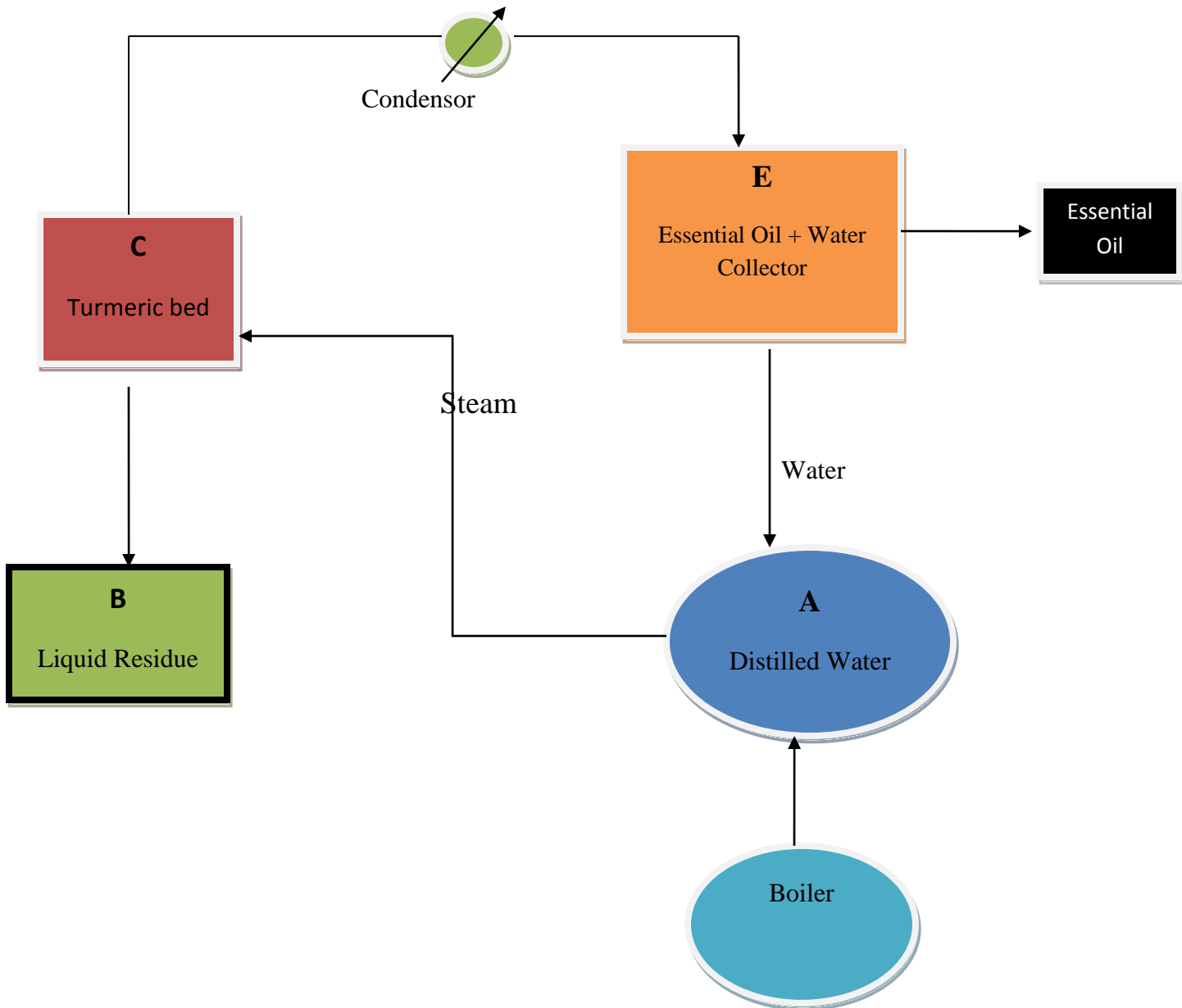


Figure 2: semantic of experimental setup

3.5 Yield Estimation of Turmeric Oil

Essential oil yield from rhizomes of turmeric was estimated using following expression.

$$\% \text{ oil Yield} = \{ \text{Essential oil obtained} * 100 \} / \{ \text{Fresh feed weight} * (1 - \% \text{moisture content} / 100) \}$$

3.6 Estimation of Percentage moisture Content

The Chopped turmeric was kept in oven at 105 degree Celsius for 4 hours. The loss of the weight gives the %moisture content.

$$\% \text{ moisture content} = [\text{Initial weight} - \text{final weight (after drying)}] * 100 / \text{Initial weight of sample}$$

3.7 Analysis of Essential Oil

Gas Chromatography- Mass Spectroscope was used for the analysis of turmeric oil (rhizomes) obtained experimentally. Carbowax 20 capillary column was used. The oven temperature were kept in between 60-260 degree Celsius at the rate of 3 degree Celsius/5min. Nitrogen gas was used as carrier gas. GC-MS Analysis was carried out at SAI lab, Thapar University, Patiala

The GC-MS results were analyzed by the comparison of mass spectra fragmentation pattern (m/z and relative abundance), retention time, and molecular weight with that library data.

Chapter 4

RESULT AND DISCUSSION

The fresh turmeric were chopped and fed to the column of the experimental setup for the production of essential oil. The all the experiments were performed at normal room conditions. The room temperature were observed in between 20-30 °C during the experiments.

The all the experiments were performed at atmospheric pressure.

4.1 Moisture content

The moisture content of the raw turmeric were estimated and given in table 1 at room temperature 25-30 °C. Total seven samples (S1 – S7) of chopped turmeric rhizomes were taken for moisture estimation. The weight of each sample (S1-S7) is 50gm. The Chopped turmeric was kept in oven at 105 degree Celsius for 4 hours. The moisture content of fresh turmeric were obtained in between 75.2-77.2 % at room temperature and normal atmospheric pressure. Hence, the average value is considered as 76.02% moisture content.

Table 1: %moisture content in different sample of same weight

<i>Sample Number</i>	<i>% Moisture content</i>
S1	75.4
S2	75.7
S3	75.2
S4	75.9
S5	75.8
S6	77
S7	77.2

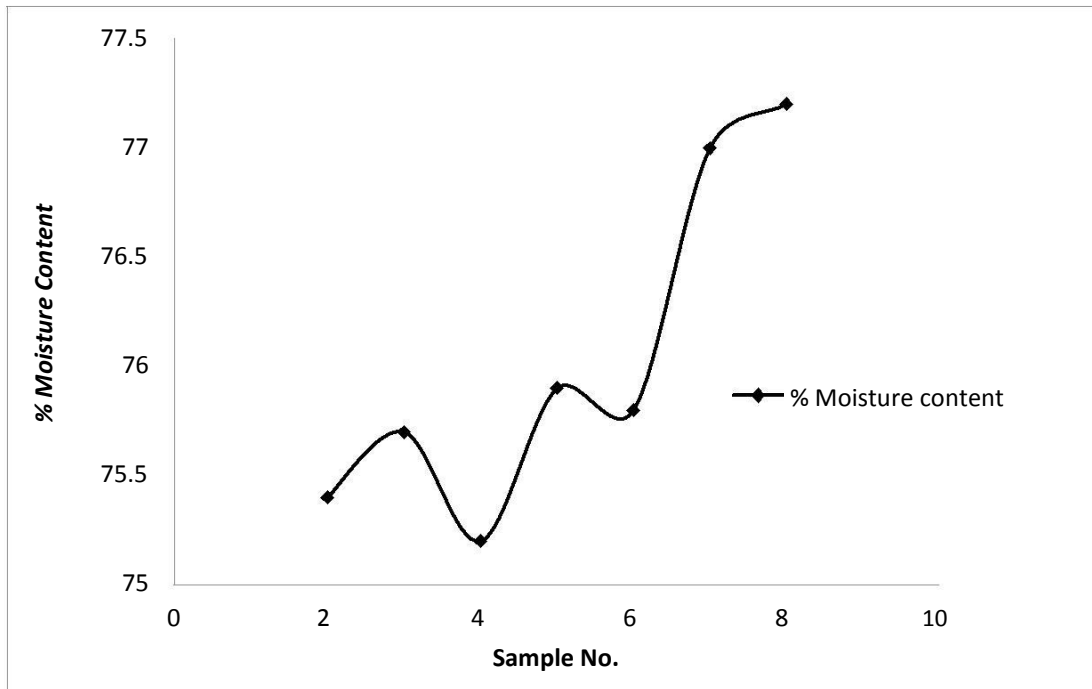


Figure 3: variation of moisture contents in different samples

4.2 Turmeric oil yield

The essential oil yield from rhizomes of turmeric is estimated using the following expression

$$\% \text{ oil Yield} = \frac{\{\text{Essential oil obtained} * 100\}}{[\text{Fresh feed weight} * (1 - \% \text{ moisture content} / 100)]}$$

The sample from S8 to S16 was used to optimize the operation time of the distillation process. The weight of each sample (S8-S16) was 500gm. The variation in the % yield of turmeric oil with operation time is given in Table 2 and graphically shown in Figure 4. The Figure 4 shows that the turmeric oil yield is continuously increases with increase in the time up to 180 min. After 180 min operation of sample, S13, the oil yield became constant. Furthermore, the samples, S14-16 were operated more than 180 min but the oil yield is almost constant. The details of oil yield with time variation is given in Table 2 and the data are plotted in Figure 4. In Figure 4, the % oil yield is plotted verses time up to maximum 270 min. It is seen that the % oil yields become constant after

180 min. with constant yield of 2.5%. Hence, 180 min is considered as an optimum time for these set of experiments.

Table 2: Variation of % oil yield with operation time

Sample No.	Sample No.	% Oil Yield
S8	30	0.31
S9	60	0.54
S10	90	1.10
S11	120	1.64
S12	150	2.07
S13	180	2.50
S14	210	2.50
S15	240	2.50
S16	270	2.50

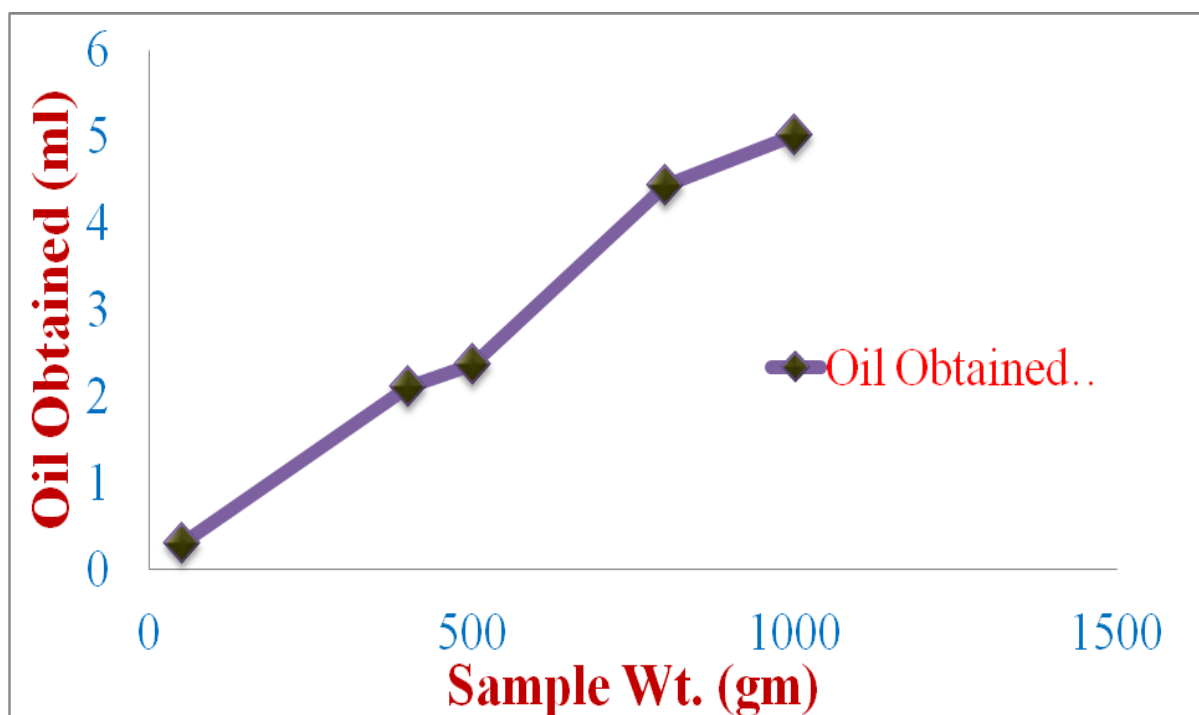


Figure 4: Variation of turmeric oil %yield (v/w) with time

Table 3: Turmeric oil (ml) with increasing weight of samples

Sample Number	Sample Weight (gm)	Oil obtained (ml)	% yield (v/wt)
S17	50	0.30	2.50
S18	400	2.11	2.20
S19	500	2.37	1.98
S20	800	4.43	2.31
S21	1000	5.01	2.09

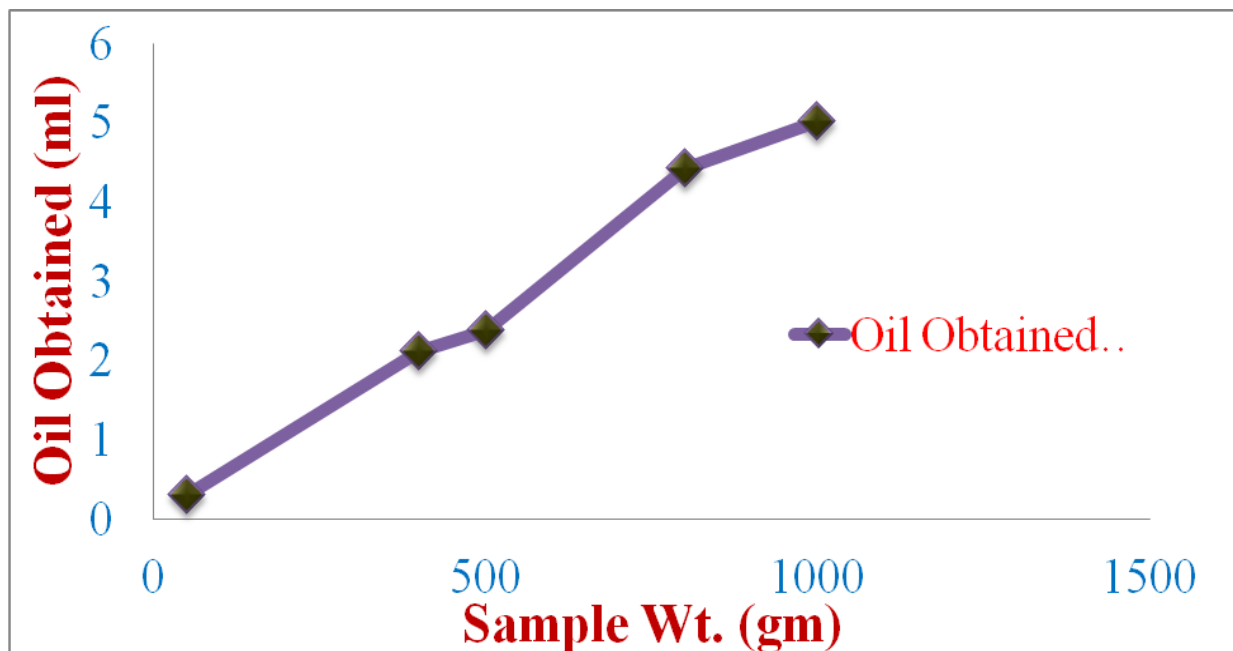


Figure 5: Variation of Sample weight (gm.) vs. oil obtained (ml)

The turmeric oil obtained from 5 samples (S17-S21) is tabulated in Table 3. In the sample S17 to S21 the rhizome weight has been increased from 50 gram to 1000 gram. The tabulated values (Table 3) are plotted in Figure 5. The observation of Figure 5 shows that the obtained oil is increasing with increasing weight of rhizomes which is obvious, but the significant amount of increased in yield were observed using the present method over conventional one. The total yield obtained from the rhizomes of *Curcuma longa* L. was in range 1.98-2.50 % (on dry basis) respectively.

4.3 Identification of major compounds using GC and GC-MS

GC-MS of rhizomes oil was carried out at SAI Lab, Thapar University, Patiala. The identification of the compounds present in the oil mixture has been done by comparing mass spectra fragmentation pattern and retention time with the library values.

Table: 4 Identified compounds present in turmeric oil, water and mixture.

Turmeric oil	WATER	Residue mixture
1,8-cineole	Beta turmerone	Zingiberene
Beta-cedrene	Ar.- Turmerone	L-Phellandrene
Terpan	Alpha curcumene	Turmerone
Alpha.- Terpinolene	Cinnamyl Tiglate	Farnesol
Terpan	Cedrene	Paracymene
		ar curcumene
l-phellandrene	caryophyllene	
		2-Methyl-Nonane
ar curcumene	A-Phellandrene	
Alpha.-terpinilene	L-Phellandrene	Para-cymene
Para-cymene		Nonane, 2-Methyl
	Alpha-thujene	
	Cycloheptane	Cyclohexane, Methyl
Turmerone		
	Zingiberene	Methylcyclohexane
Beta Sesquiphellandrene		
curlone	Beta sesquiphellandrene	Sextone B
zingiberene	(+)-2-Caren	

GC-MS of rhizomes oil was carried out at SAI Lab, Thapar University, Patiala. In GC-MS 20 mass spectra were obtained. Identification of compounds has been done by comparing mass spectra fragmentation pattern and retention time. GC-MS analysis of rhizome oil sample was carried out at Thapar University, Patiala. In GC-MS analysis about 20 compounds were obtained. GC followed by GC-MS analysis of rhizome oil sample was carried out at SAI Lab, Thapar University, Patiala. In GC-MS analysis only 20 mass spectra's were obtained. Identification of the compounds has been done by comparison of mass spectra fragmentation pattern and retention time. The identified

compounds are ar-curcumene (2.3%), zingiberene (4.01%), dehydrocurcumene (2.0%), ar-termerone (15.8%), termeroan (4.4%), curlone (5.6%), and others (Table 4) some of them are still unidentified. And many are unidentified compounds. Unidentified volume consist approximate 68 compounds which has to be identified. The detailed analysis, GC-MS spectra are given in Appendix 1.

Table 5: Identified compounds with their compositions are as follows:

<i>S. No.</i>	<i>Identified compound</i>	<i>Identified compound</i>
1	ar-turmerone	15.8%
2	Curlone	5.6%
3	Termerone	4.4%
4	Zingiberene	4.01%
5	ar-curcumin	2.3%
6	Dehydro curcumene	2.0%
7	Unidentified	65.49%

Present modified design of the setup shows that a continuous separation of essential oil from turmeric rhizome is possible as more efficient compared to conventional processes in use also this being an Eco-friendly process for essential oil separation from plant materials can also be used to minimize oil losses.

CHAPTER 5

CONCLUSION

In present investigation the emphasis was laid on the feasibility of the modified distillation process to obtain essential oil from plant origins. A new design has been used for the entire experimental work. The modified design used in present studies can be used efficiently for obtaining essential oil from other plant materials/spices. The oil yield obtained from rhizomes ranges between 2.38 - 2.48 % (v/w). The obtained yield is 13% more in rhizome oil.

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Appendix -1: GC of turmeric oil

