SUPERCRITICAL FLUID EXTRACTION OF NATURAL MEDICINAL PRODUCTS

THESIS SUBMITTED

BY

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JADAVPUR UNIVERSITY

Kolkata – 700 032, West Bengal, India

YEAR 2019

JADAVPUR UNIVERSITY

KOLKATA-700 032, INDIA

INDEX NO. : 29/12/E

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II. Roy, S., Guha, C., Saha, A. K., Sahu, S. J., 2019. Supercritical Carbon Dioxide Extraction of Clove (Syzygium aromaticum) Bud Oil in an Annular Grate Feed Contactor: Effect of Axial to Radial Surface Enhancement Factor. International Journal of Innovative Technology and Exploring Engineering (IJITEE). 8(6), 290-301.

III. Roy, S., Guha, C., Saha, A. K., 2016. Studies on Reduction of Extraction Time of Supercritical CO_2 to Save Energy. IJRET: International Journal of Research in Engineering and Technology. 5(1), 22-25.

Conference Publication:

I. Roy, S., 2015. Supercritical Fluid Extraction : A Green Extraction Technology to Protect Environment. National Conference on GREEN ENERGY,NCGE-2015.

II. Roy, S., Guha, C., Saha, A. K., 2015. Studies on Extractor Bed Geometry on Supercritical Carbon-dioxide Extraction of Clove Oil. Chemcon, 2015.

4. List of Patents: None

5. List of Presentations in National/International/Conference/Workshop:

I. Roy, S., 2015. Supercritical Fluid Extraction Technology : A novel promising technology for the production of Solid-Lipid Nanoparticles. International Conference on Nanotechnology ICNT - 2015.

II. Roy, S., 2015. Supercritical Fluid Extraction : A Green Extraction Technology to Protect Environment. National Conference on GREEN ENERGY, NCGE-2015.

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CERTIFICATE FROM THE SUPERVISORS

This is to certify that the thesis entitled "**Supercritical Fluid Extraction of Natural Medicinal Products**" submitted by **Mrs. Sutapa Roy** who got her name registered on 06/07/2012 for the award of **Ph. D. (Engineering)** degree of **Jadavpur University** is absolutely based upon her own work under the supervision of **Prof. (Dr.) Chandan Guha** (Department of Chemical Engineering, **Jadavpur University**) and **Prof. (Dr.) Asit Kumar Saha** (Principal, **Haldia Institute of Technology**) and that neither her thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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……***Dedicated***

TO

MY BELOVED PARENTS,
LOVELY HUSBAND,
&
LITTLE DAUGHTER…

ACKNOWLEDGEMENT

I take this opportunity to convey my sincere gratitude and thanks to the idols of my academic career Prof.(Dr.) Chandan Guha, Chemical Engineering Department, Jadavpur University and Prof. (Dr.) Asit Kumar Saha, Principal, Haldia Institute of Technology for their being kind enough to thoroughly guide me throughout the course of my research work.

My sincere thanks go to the Indian Institute of Chemical Engineers (IIChE) Kolkata for their partial financial assistance and I would also like to thank the H'ble Vice Chancellor of Jadavpur University for providing me necessary permission to complete this work in this esteemed University.

I also extend my sincere gratitude to Prof. (Dr.) Chiranjib Bhattacharjee (Dean FET, Jadavpur University) for his encouragement, valuable suggestions and support throughout my work.

I convey my thanks to the Department of Chemical Engineering, Haldia Institute of Technology for providing the laboratory facilities throughout my work. I cordially acknowledge the immense support that I had been bestowed by the faculty members and staffs of chemical engineering department of Haldia Institute of Technologyto complete the work.

I also owe a special thanks to Dr. Mukesh Singh of Department of Biotechnology, Haldia Institute of Technology for rendering his valuable knowledge and support in the field of antioxidant and antimicrobial studies for my research work.

I am personally amply grateful to Mr. Sridam Das (Department of Chemical Engineering) & Mr. Debasis Maity (Department of Production Engineering) of Haldia Institute of Technology and Ms. Misti Das for their kind co-operation and support.

Thanks are also due to the Department of Food Technology, Haldia Institute of Technology and M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India for providing their laboratory facilities during my research work.

I also thank all my friends who provide their help and support as they can.

I acknowledge that I am much indebted to all family members particularly my husband and daughter for their love and support in my academic pursuit.

Above allI would extend the deepest sense of regards to my dear parents for their continuous care, supports and encouragements to finish any work. I dedicate the thesis to them.

The lord has provided me the strength to complete this work; insignificant through it is, against my limitations and through great hardship. To Him I thank everything and ask for His blessing.

SUTAPA ROY

CONTENTS

LIST OF NOMENCLATURE/ ABBREVIATIONS/SYMBOLS

2FI	2 factor interaction
%OY	Percentage oil yield
A	Proportionality constant of Dynamic mathematical modeling
ANOVA	Analysis of variance
ARSEF	Axial to radial surface enhancement factor
B1	Cylindrical extractor bed
B2	Annulus extractor bed with inner channel of 0.75mm diameter
B3	Annulus extractor bed with inner channel of 1.5mm diameter
β_0	Regression coefficient of intercept
β_1, β_2, β_3	Regression coefficients for linear fit
β_{12}, β_{13}, β_{23}	Regression coefficients for 2FI fit
β_{11}, β_{22}, β_{33}	Regression coefficients for quadratic fit
CCD	Central composite design
CER	Constant extraction rate period
CO_2	Carbon dioxide
D_P	Particle size
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl
E	Extractor
EO	Essential oils
F	Mass of feed loaded
FC-CCD	Face-centered central composite design
FCR	Folin-ciocalteu reagent
FER	Falling extraction rate period
FICA	Fe^{2+} Ions Chelating Assay
FRAP	Fe^{3+} Reducing Antioxidant Power
GAE	Gallic acid equivalents
GCMS	Gas chromatograph mass spectrometer
HAT	Hydrogen atom transfer
K	Rate constant

L	Length of the extractor bed
LPIA	Lipid Peroxidation Inhibition Assay
OEC	Overall extraction curve
P_C	Critical pressure
P	Pressure
Q_{CO2}	Solvent flow rate
r_i	Radius of inner cylinder or channel
r_o	Radius of outer cylinder
R_{CER}	The rate of extraction at CER
RSM	Response surface methodology
S I	Separator I
S II	Separator II
SCO_2	Supercritical CO_2
SCO_2E	Supercritical fluid extraction using CO_2 or Supercritical CO_2 extraction
SET	Single electron transfer
SF	Supercritical fluid
SFE	Supercritical fluid extraction
SFET	Supercritical fluid extraction technology
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reactive species
T	Temperature
T_C	Critical temperature
TPC	Total phenolic content
t	Time
t_{CER}	Constant extraction rate period
t_E	Extraction time or Period of extraction
t_S	Static period of extraction
Y	Amount of oil extracted expressed as (%OY) at time t
Y_∞	Measure of the maximum value of Y after infinite time
Y_{CER}	% yield achieved at CER

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PREFACE

The present thesis is written as the final thesis for the doctoral degree in Engineering, based on the investigation entitled "**Supercritical Fluid Extraction of Natural Medicinal Products**". The conventional cylindrical geometry of the extraction vessel was replaced by annulus bed geometry. A face-centered central composite design developed under Response Surface Methodology was used to optimize the process variables such as extraction pressure, extraction temperature, extractor bed geometry, and particle size, of supercritical fluid extraction. Dried clove buds and turmeric rhizomes were used as raw material to investigate the bed geometry influence on the extraction process. The yield of oil and its major bioactive compounds both were observed to increase in the annulus bed as compare to the cylindrical bed of supercritical extraction unit.

Hence, the annulus bed geometry for supercritical CO_2extraction can be used to extract the essential oil from plant biomass as it reduces the mass transfer resistance and induces the turbulence inside the bed, that contribute to the mass transfer rate enhancement. For designing this particular extractor, the channelling effect must need some special care. I hope that the findings of the research will be equally worthful for researchers, extension workers, and food and pharmaceutical industrialists as well.

The entire works were presented here in the following chapters: Chapter I is the introduction part which explains the background of the research topic such as the pharmaceutical importance of natural essential oil, different extraction methods used for separation of oil, and advantages of supercritical CO_2 extraction technology. This chapter justifies the reason for choosing this research topic. Chapter II, the literature review, deals with the important work done in the past and finding out the research gap. It provides a conceptual frame work for carrying out the research work as well as understanding the outcome of present work. Chapter III, describes the objectives of the research work with hypothesis. Chapter IV, is the materials and methods deals with the raw materials, analytical chemicals used in this work and describes the experimental procedures of supercritical fluid extraction and Soxhlet extraction. Chapter V, This section deals with procurement of experimental material, sample preparation and details of methodology used in this research work to optimize the process parameters for clove oil and turmeric oil extraction.. Chapter VI is the biomedical testing procedures applied to find the antioxidant properties of clove extract. Chapter VII containing results and discussion deals with the results of this research work. Research outcomes are illustrated with the help of tables, figures, and statistical model equation and the discussion are made to explain the results obtained with appropriate reasons and support. Chapter VIII is the summary and conclusion gives brief description of the results of the investigation and the conclusion drawn from this investigation. References deals with citation which has been consulted during the course of investigation.

CHAPTER 1

INTRODUCTION

1. Introduction

Nowadays, it is hard to find any process industry in the field of food, beverage, medicine, cosmetic, perfume, bio-fuel, or fine chemicals synthesis, which is not related with the extraction process. The industrial extraction processes require large quantities of solvents and at least 50% of the total energy consumption of the whole industrial establishment [Chemat, 2012]. The interest on supercritical fluids (SFs) is growing exponentially since 1980s after the establishment of SFs as real green alternative solvents to the conventional organic solvents which are mostly flammable, volatile and toxic in nature and have some negative impact on environmental pollution and global warming. Today, SF based technologies are proven as sustainable bio-chemical engineering technologies in a wide range of industrial applications for their zero or less use of hazardous organic solvents, production of natural products free of contaminations and reduction of environmental threats. Extraction of various valuable products (simple as well as complex molecules) from natural resources is the most developed field of SF based technologies on an industrial angle. The success of the process of supercritical fluid extraction (SFE) depends on the optimization of the operating parameters. The process optimization influences several things, such as the quantity and quality of extract; systematic design and analysis of the extraction unit to meet high-pressure operational safety; appropriate sizing of the pieces of equipment to increase extraction efficiency. In an extraction module, extractor is the principal element where actual extraction or recovery process is carried out under a condition of high pressure (above critical condition). Since variation in geometry of the extractor may lead to either positive or negative impact on the extraction process, the present research work is intended to introduce a unique design concept of an extractor to synthesize essential oils from various parts of plants with the help of supercritical carbon dioxide (SCO_2) as solvent and its influence on the performance on SFE process is verified comparing with the performance of conventional extractor for the same extraction processes. Before exercising the details design and performance of the SF extractor, natural medicinal products particularly, essential oils, their applications, various methods of essential oils extraction, and mechanisms of action against destructive cell-damaging free radicals are discussed in this section as a background of this research.

1.1. Natural Medicinal Products:

Natural medicinal products are a range of bioactive chemical compounds or substances extracted or synthesized from various parts of plant or animal sources. This diverse group of bioactive compounds has some beneficial effect on human health [Halliwell, 2007]. Their mechanism of action on a living organism, tissue or cell may be like an antioxidant to prevent cell-damaging free radicals or cell repairing agent to recover from oxidative stress or immunity-enhancing agent to decrease the risk of diseases [Halliwell, 2007]. Some important bioactive compounds are polyphenols, phytosterols, fatty acids, non-pro-vitamin A carotenoids, and peptides [Astley, 2016]. Indigenous peoples and ancient civilizations used these natural medicinal sources as drugs in their crude form. Now extracts in a more concentrated form of these natural active ingredients lead to the development of modern “natural medicines” to compete with or replace the “synthetic medicines”. Thus a large number of recommended drugs of present civilization are directly or indirectly derived from the natural resources and the consumer’s preference for “natural medicines” becomes a trend due to their growing awareness about the benefit of natural products.

1.2. Essential Oils:

Essential oils are complex liquid mixtures of many natural bioactive chemical compounds that are highly volatile in nature and synthesized in concentrated form from most aromatic and medicinal plants. These oils can affect the sense organs by their typical smell and aroma generated naturally from volatile matter. The aromatic compounds of essential oils can be isolated in a dense form applying a physical technique or operation from roots, rhizomes, stems, bark, leaves, buds, flowers, fruits, seeds, resin, woods and balsam of a wide variety of plant species [Mukhopadhyay, 2000]. Other popular names of essential oils are ethereal oils, volatile oils or aetherolea. The main organic constituents of essential oils include terpenes, aldehydes, phenols, ketones, alcohols, coumarins, lactones, ethers, and esters [Bakkali, 2008; Westover, 2010]. The bioactive components of essential oils have numerous therapeutic benefits for their antioxidative, anti-carcinogenic, antimicrobial, antibacterial, antiprotozoal, antiviral, antifungal, anti-inflammatory, anti-diabetic, antivenom, anti-mutagenic, antifibrotic, antispasmodic, antidepressant, anesthetic, febrifuge, anticoagulant, antiseptic, antiulcer, immunomodulatory, astringent and analgesic properties. Today the development of essential oils based industries have gained momentum as essential oils are recognized as low-volume high-value oils since the application of a small dose of high quality oil can provide benefits in large scale. The important applications of essential oils are listed as follows [Chamorro, 2012].

i. Flavoring and coloring agents of food, beverages, and confectionery industries
ii. Food preservatives
iii. Pharmaceutical aids
iv. Aroma chemicals in aromatherapy
v. Cosmetic and perfumery ingredients
vi. Green pesticides
vii. Insect repellents
viii. All purpose cleaner to naturally disinfect
ix. Room refreshing agent etc.

Based on the usage of the essential oils, their production statistics were reported by Hunter, M., (2009). This is shown in Fig.1.1. Because of this wide range of applications, the commercial growth of essential oil production industry is increasing worldwide annually at a rate of approximately 8-10% [Hunter, 2009]. Thus, there are different interests of essential oil based researches that need to be explore, such as, development of the extraction techniques, isolation of bioactive components of potential interest in purer form, searching of more and more essential oil bearing plant species from world's vast plant kingdom, and firming these wild species collecting from their origin for the expansion of the world market of natural product to meet the consumers high demand. India leads in this market of the natural product as one of the world's largest producer, consumer, and exporter of essential oils [Baser, 2015; Barbieri, 2018].The climatic conditions and soil quality of India favour the agricultural growth of aromatic plants. Government policies also drive the continuous development of science and technology. Moreover, the huge

investment in the production and trading of essential oils make India play the dominant role in the world market of aroma producing plants and essential oils.

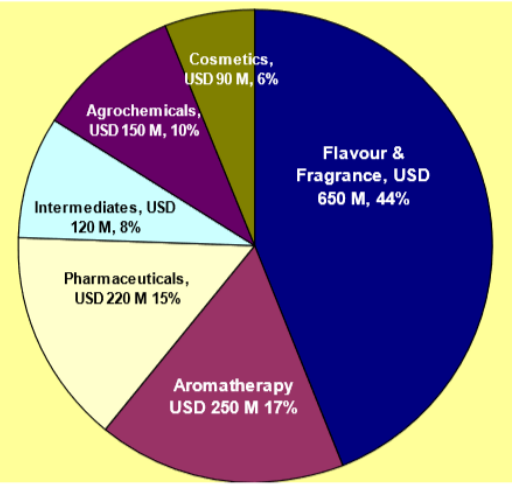

Fig1.1: World Farm-gate Value of Essential Oil Production (USD) (Source: Hunter, 2009)

1.3. Methods of Extracting Essential Oils

Several methods are available to extract essential oils from various parts of a plant species. The selection of extraction method for the recovery of bioactive components of essential oils plays an important role on the yield and distribution of chemical constituents of crude extract which lead to the variation of the quality of the product greatly. Reviewing the broad area of application of essential oils in aroma, pharmaceutical, food and cosmetic industries and growing health awareness of the consumers of developed countries, sometimes it becomes principal consideration to choose quality over quantity of products. It is reported that use of less-quantity high-quality essential oil is more beneficial than a large amount lesser-quality product. This indicates that the choice of extraction method also depends on the intended application of the final yield. Some methods of extraction are in use for several decades and termed as traditional or conventional methods. Some other methods developed later to meet the objectives of green, sustainable technologies i.e. replacing harmful organic solvents by alternative solvents like CO_2 or lowering energy consumption or reducing time of production or minimizing steps of operation [Anastas, 2010; Rombaut, 2014]. All these methods are listed below-

a. Effleurage
b. Solvent-Extraction
c. Cold Pressing or Expression
d. Steam distillation Method
e. Hydro Distillation or Water Distillation
f. Super Critical CO_2 Extraction

g. Ultrasound-assisted extraction

h. Microwave-assisted extraction

❖ **Enfleurage**

Enfleurage is a traditional method used to extract essential oils from scented flowers. The principle of high absorption power of fat for odor bearing molecules is applied. The petals of flowers are spread over the top surface of a specially prepared fat surface where the fragrant petals are allowed to emit their perfumes for a period of 24hrs or longer depending on the nature of flower (Fig. 1.2). Finally, the oil-saturated fat layer is washed with alcohol, which is distilled under vacuum to isolate the scented essential oils [Handa, 2008].The essential oils produced by this method has more natural odor due to the absence of heat load.

Fig. 1.2: Enfleurage tray with flowers.
Source: https://artisanaromatics.com/what-are-enfleurage-essential-oils/

❖ **Solvent Extraction**

Solvent extraction is a conventional technique to isolate soluble components from plant matrices with the help of the dissolving power of hydrocarbon solvent applying heat or agitation to enhance mass transfer. For recovery of essential oils, plant materials are chopped or crushed. The extractor unit is then loaded with plant material with the help of perforated trays and continuously contacted with a suitable solvent. Volatile oils present on the surface of the solids dissolve quickly in the solvent. For dissolving more soluble matters, the solvent penetrates into the interior of the solid matrix from where soluble solutes are diffused out. At the end of extraction, the solute rich solvent is filtered and treated to separate the solvent from the soluble matter and thus concentrated yield is obtained [Zhang, 2018].

Selection of a suitable solvent is the key to the success of this process. Selectivity, solubility, polarity, recoverability, chemical reactivity, vapour pressure, safety, health hazards, cost, and availability are some essential factors that need to be considered during the selection of solvent. The particle size of the plant materials, solvent-sold feed ratio, temperature, and time of extraction are some other features that have an influence on this extraction process.

Solvent extraction is advantageous than distillation w.r.t. the applied heat load. Due to moderate operation temperature (around 50^0C), loss of thermolabile compounds is less in this method. As a result, the fragrance of the final product is better than any type of distillation. However, the methods of solvent extraction are challenging to control.

❖ **Cold Pressing or Expression**

Cold pressing or expression is a mechanical extraction method to process mainly citrus fruits – lemon, orange, lime, grapefruit, bergamot, petitgrain, and tangerines for recovery of essential oils. The extraction by cold process helps to preserve the natural aroma and potential benefit of these oils. Historically in this method, the peels of citrus fruits are milled or chopped to rupture the essential oil glands and then pressed by hand against a hard surface which is placed under a sponge that absorbs the plant extract. Once the sponge is loaded with the mixture of essential oil, water and/or juices, the essential parts are recovered by squeezing the sponge into some container. However, use of the hand is impractical for large scale application. Different machines are designed to achieve the same effects for commercialization (Fig. 1.3). Centrifugal forces are also employed to recover the citrus oils in most modern cold press devices. The main disadvantage of this method is that the shelf life span of the product is relatively short [Martı´nez, 2008].

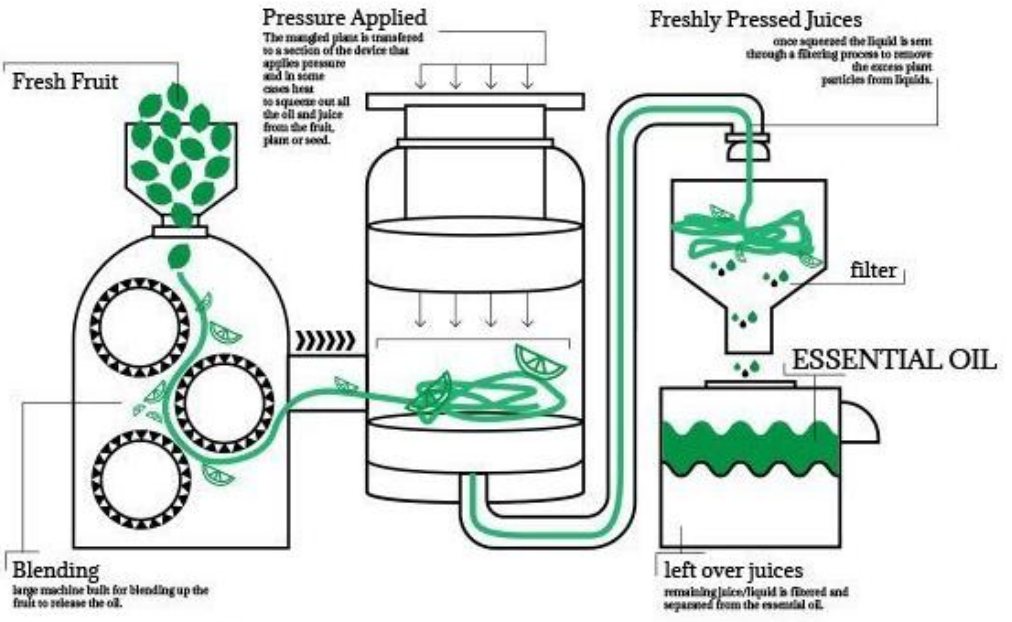

Fig 1.3: Essential Oil Recover by Cold Expression (https://sanatherapeutics.com)

❖ **Steam Distillation**

Steam Distillation is popularly used ancient method to produce essential oils from aromatic plants. In this method, the aromatic plant materials are loaded over a perforated grid placed above the steam inlet nozzle of a large still pot (Fig. 1.4).A live steam generated outside the still is injected into the vessel and passes through the supported plant material. The steam helps the aromatic oil molecules to come out to the surface of the material breaking the oil glands as well

as supplies energy to vaporize the oil molecules. The mixture of vapor and steam goes to a condenser for liquefaction with the help of a cooling water system, and condensed oil and water drops are collected in a separator where immiscible oil-water forms two separate layers.

Advantage of this method is that as steam is generated outside the still, its temperature or pressure can be readily controlled. Thus this technique can be carried out successfully under varying pressure depending on the aromatic oils extraction difficulty [Rassem, 2016].

For steam volatile oil molecules, high boiling point essential oils and hardy plant materials like bark, wood etc., steam distillation is more effective (Tandon, 2008). In this method of distillation, the aroma compounds of essential oils whose boiling points range from 150^0C-350^0C can be vaporized at a lower boiling point around 100^0C than their individual boiling point. The principal mechanism is that when their cumulative vapor pressure becomes equal with the atmospheric pressure, all the oil molecules start to boil (Chamorro, 2012).

One important drawback of this method is the much higher capital investment for the establishment. This method of extraction is not suitable for water soluble, highly volatile, and thermolabile components [Sarker, 2005].

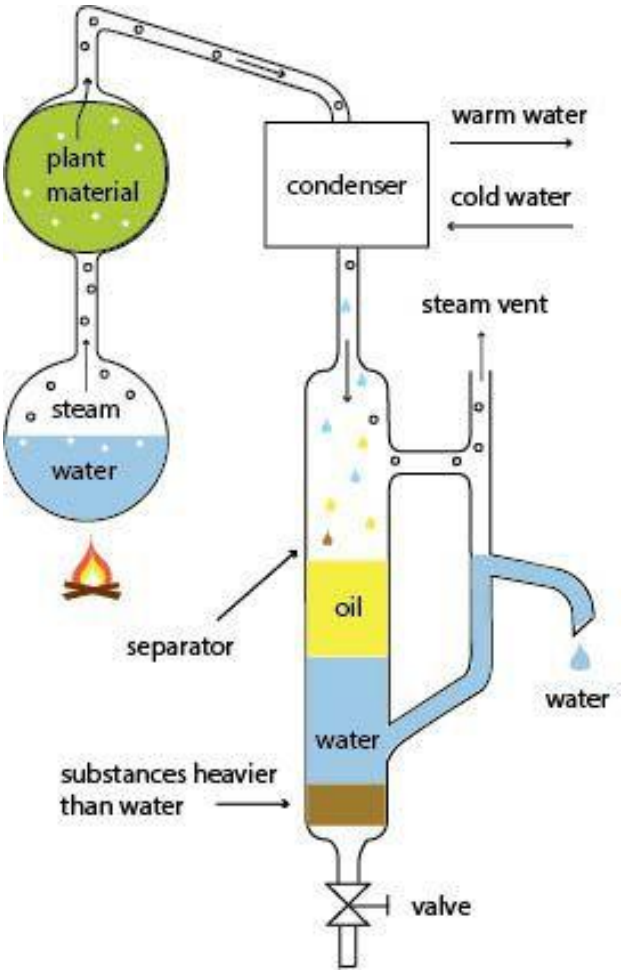

Fig. 1.4 : Essential Oil Extraction by Steam Distillation
(https://www.pinterest.com/pin/228135537350517432/)

❖ Hydro Distillation or Water Distillation

Hydro-Distillation is one of the oldest methods, traditionally used to extract essential oils easily from a whole plant or plant parts [Meyer-Warnod, 1984]. It is also known as water distillation for the use of water as an extracting medium or solvent. In this method, the plant materials are packed in a large still filled with sufficient water to submerge the material (Fig. 1.5). This method is suitable for finely chopped plant material or their dried ground form. It is useful for hardy plant parts like wood, root, bark, nuts etc. With the help of a heat source, water is boiled to produce vapor. During this process, plant materials need to be stirring continuously to prevent the settling of dense plant matter to the bottom of the still where heat is supplied. It may cause thermal degradation of the dense matter during long thermal exposure [Handa, 2008]. Boiling water itself can induce motion of the plant materials if they are loaded loosely. Thus penetration of solvent through plants matrix is easier in this method than steam distillation in which plant materials form lump through which live steam cannot go through [Handa, 2008].Under heating load, essential oils expand in the oil glands and rupture the cell barrier and mix with the solvent. Volatile oil molecules along with steam come out and pass through a condenser to liquefy the vapor mixture. Drops of immiscible water and oil are collected in a decanter from where oil layer is separated from the water layer. The residual water sometimes may carry the odour of essential oils and treated as a by-product. People refer it by several names such as hydrolate, hydrosol, essential water, herbal water, aromatic water, floral water, or herbal distillate.

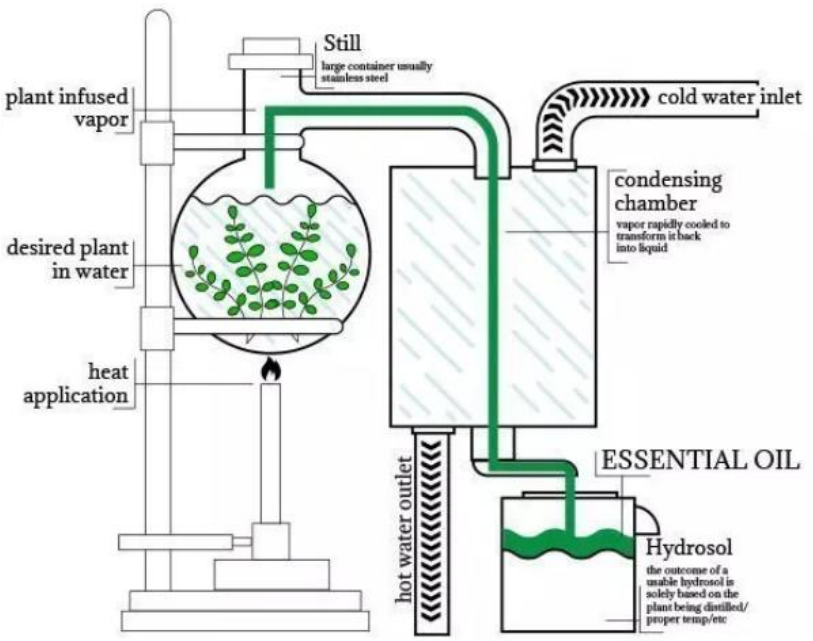

Fig. 1.5 : Essential Oil Extraction by Water Distillation (https://sanatherapeutics.com)

The amount of extract by hydro or water distillation depends on mainly water solubility of the oil molecules. Other factors that need to be considered are the weight of feed plant mass, feed to solvent ratio (mass/volume), size of raw material and nature of aromatic plant material [Parikh, 2011].

The distillation time depends on the nature of plant material being processed. Prolonged distillation may produce only a small amount of essential oil, but does add unwanted high boiling point compounds and oxidation products.

Expenditure of this method is less than steam distillation. Process of extraction is slow compared with other distillation methods. The main drawback of this method is incomplete extraction i.e. poor yield and extraction of unwanted matter due to large extraction time [Handa, 2008]. Product quality may deteriorate by polymerization of certain sensitive bio components like aldehydes during prolonged exposure or hydrolysis of the required products like esters [Handa, 2008; Dilworth, 2017]. High fuel cost, larger space requirement, and a greater number of extraction vessels are some other disadvantages of this method. For this reason, the economic feasibility of this traditional method is very poor. This process is used mostly combining with the injection of steam or when due to the nature of plant materials steam is not suitable for extraction [Handa, 2008].

❖ **Water and Steam Distillation**

Combined water and steam distillation are similar to water distillation; however, the material in question is not in direct contact with water but rather placed on a solid support above the boiling water so that steam can directly pass through it. For best yields, the material should be evenly distributed so that there is efficient contact with the material by steam. Some setups increase their efficiency by addition of a cohobation tube which ensures that after condensation and separation of the essential oil, water is returned to the still for reboiling, thereby ensuring that there is enough water in the system to ensure complete extraction. This method also ensures minimal loss of oxygenated components, including phenols. Compared to water distillation, this method gives higher yields, better quality oil, and the process is quicker than water distillation.

❖ **Supercritical CO_2 Extraction (SCO_2E)**

One of the most advanced technologies, supercritical fluid extraction (SFE) using low cost CO_2 as a greener solvent above its critical condition is growing with a faster rate in the extraction space to meet the objective of reduced use of organic solvents in the extraction of natural products. Supercritical fluid (SF) or dense-gas acquires the powerful solubilization property of liquid solvent in addition to the advantageous transport properties of gases [Wenclawiak, 1992.]. This procedure requires lower extraction temperature than conventional extraction processes like solvent extraction, hydrodistillation, and steam distillation. It allows pressurized carbon dioxide to extract natural substances from plant or solid matter loaded inside the specially designed high pressure vessel/s (Fig.1.6). The great importance of SCO_2E method is because of the production of high quality products that bear the properties like essence, therapeutic benefit, etc., as close as possible to the original plant extract. Low to moderate operation temperature allows this extraction process to maintain the integrity preservation of thermolabile compounds [Mendiola, 2007]. The gaseous state of CO_2 at ambient condition and high selectivity of as a solvent leads to the formation of solvent-free extract in a more concentrated purer form, which are the most attracting factors for this method. This method provides oxygen-free extraction condition, minimizes extraction period and solvent consumption, and reduces the subsequent secondary solvent recovery step and related cost of solvent recovery [Began, 2000]. Collection of the extracted analytes from solvent phase is very much crucial from the economic point of the

process as separation step most of the cases involve significant loss of the high-value volatile analytes that lead to the poor efficiency of this novel process. The high installation cost of the extraction unit due to high pressure operation and weak polarity of the solvent CO_2 that does not favour the extraction of polar solutes are considered as main drawbacks for this process.

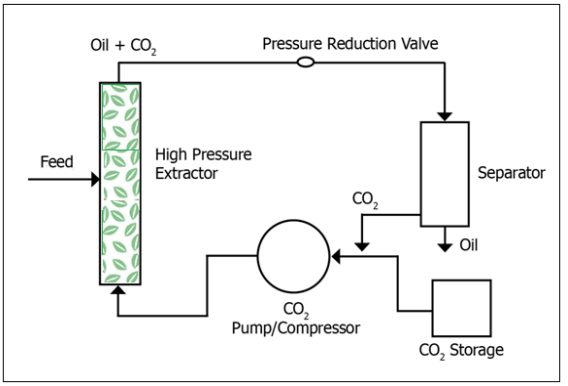

Fig. 1.6 : Flow Diagram of a Typical Supercritical Carbon Dioxide Extraction Unit

❖ **Ultrasound-assisted Extraction (UAE)**

Ultrasonic-assisted extraction (UAE) or ultrasonic extraction or sonication is an emerging technology applicable for the extraction of thermolabile and unstable compounds from natural resources. Other advantages of this technique are mainly reduced extraction time; and low solvent and energy consumption [Zhang, 2018]. The ultrasound technology using ultrasound Probe or ultrasound bath (Fig. 1.7) involves the use of ultrasonic wave energy that produces cavitations in the solvent. These cavitations effects improve the release of the intercellular constituents by rupturing the cell walls, the surface contact between solvents and solute, and permeability of cell walls [Dhanani, 2013]. Thus, mass transfer properties of dissolution and diffusivity as well as the heat transfer rate increase that improves the extraction efficiency of this method. This method requires the use of high-frequency ultrasound waves ranging from 20 kHz to 2000 kHz [Handa, 2008]. One deleterious effect of intense ultrasound energy on the plant active ingredients is the occasional formation of free radicals and hydrogen peroxide that makes several undesirable modifications in the bioactive molecules [Forde, 2014].

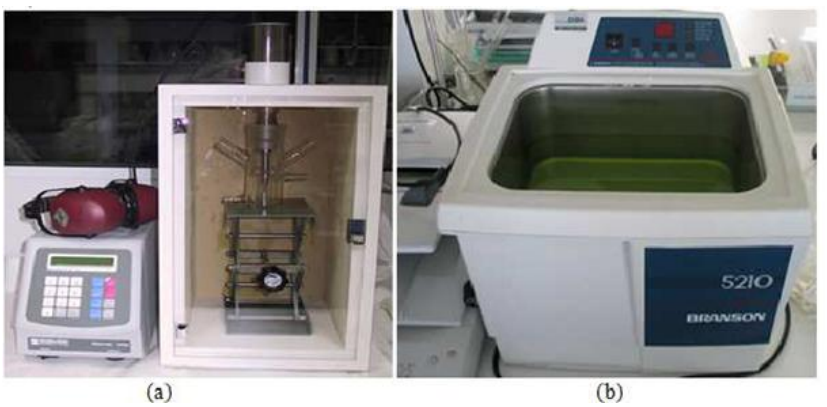

Fig.1.7: Common Type Ultrasound-assisted Extraction Unit: (a) Ultrasound Probe, (b) Ultrasound Bath (Source: Chemat, 2011)

❖ **Microwave-assisted extraction (MAE)**

Microwave-assisted extraction is an advanced extraction technology that utilizes a unique heating mechanism to reduce the extraction time remarkably (<30min) [Huie, 2002].In this method, the extraction vessel loaded with plant material is heated in a targeted and selective manner in a closed system that practically prevents any form of heat loss to the environment (Fig. 1.8). For effective heating, domestic and industrial applications microwaves are generally operated at 2.45 GHz frequency though it covers radiations of a wide range of frequencies (0.3 – 300GHz) [Camel, 2001]. Microwaves are generated from two different types of oscillating perpendicular fields, electric and magnetic fields. The principle of heating by electric fields is governed by ionic conduction and dipole rotation [Kaufmann, 2002].Heating caused by ionic conduction occurs by the electrophoretic migration of ions under the influence of the changing electric field. Polar solute/solvent molecules having high dipole-moment are facing dipolar rotation due to the alignment on the electric field. This oscillation of the polar molecules causes collisions with the surrounding molecules that release thermal energy to heat up the solution. During extraction, the microwave electromagnetic energy facilitates the partitioning of the polar solutes and disrupts the hydrogen bonding by dipole rotation and enhances the mass transfer rate by accelerating the migration of dissolved ions and promoting solvent penetration through the plant matrix. In the traditional methods of solvent extraction, the heat energy is generally transferred through the wall of the extraction vessel to the medium, and then the solvent is heated up by convection currents. Thus, it is a slow and inefficient heating process (as heat distribution is not uniform).

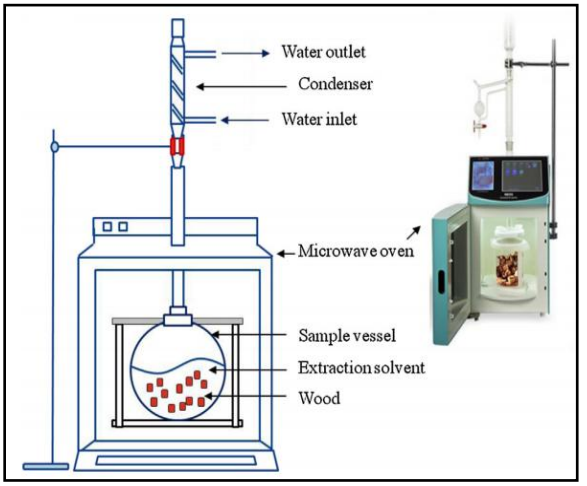

Fig. 1.8 : Experimental setup for Microwave-assisted extraction

(Source: Bouras, 2015)

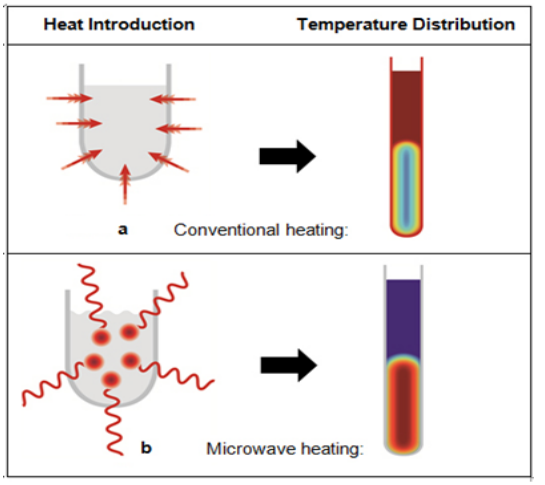

Fig.1.9: Principal of Heating and Temperature Distribution for (a) Conventional and (b) Microwave System

(https://wiki.anton-paar.com/en/microwave-assisted-synthesis/)

In contrast, microwaves irradiation results in transferring microwave energy through the vessel wall directly to all dipolar and/or ionic molecules present in the solid-solvent mixture (Fig. 1.9). The development of this advanced extraction technology was first informed by Ganzler and co-workers [Ganzler et al., 1986; Ganzler and Salgo´, 1987].

1.4. Selection of SCO_2E Method for Essential Oils Extraction

From the above study, it is clear that there are numerous methods used to extract essential oils or active components of interest. Most of these methods initially produce an extract consisting of active and inactive components that are further fractionated to obtain different targeted molecules in a purer form. Some of the conventional extraction methods used in essential oil production are often simple, cheaper, and practical. Unfortunately, that is either consumed hazardous organic solvents in a large to a moderate amount and/or required high thermal energy that may cause degradation of thermolabile molecules. Some of the processes are suffering from lager extraction time, production of a poor quality yield in terms of the presence of bioactive molecules or a large yield with a wider range of compounds, high cost associated with disposal or recovery of large amounts of organic waste, and health-safety-risk-environmental issues [Harbourne, 2013]. Thus they are considered as inefficient.

The emergence of greener technologies for extraction of natural herbal products started to develop at a faster rate since 1990s aiming reduced energy consumption, finding greener environmentally less harmful alternative solvents to replace conventional solvents, and reduced processing times [Anastas, 2010; Rombaut, 2014]. Some of the greener technologies used popularly in essential oil extraction are ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction. Summary of these technologies is given below (Table-1.1) as reported by Khaw et al. (2017).

Table-1.1 : Summary of Some Green Extraction Technology [Khaw, 2017]

Method	**Advantages**	**Disadvantages**
Microwave-assisted extraction (used with traditional methods)	rapid extraction; small amount of solvent; relatively low additional costs	use of high pressure and temperature; limited amount of sample; non-selective (large number of compounds extracted
Supercritical fluid extraction (SFE) methods	rapid extraction; small amount of organic solvent or no solvent; no solvent residue; preserves thermally labile compounds; tunable solvent (SCF) density; selective extraction (small number of compounds extracted); inexpensive to operate/run	high setup cost; technical knowledge of SCF properties required (e.g., phase behaviour, cross-over region)
Ultrasound-assisted extraction (used with traditional methods)	rapid extraction; small amount of solvent; relatively low additional cost	non-selective

Among these green extraction methods, only SFE using CO_2 as a solvent is based on the alternative solvent approach that also offers reduced processing energy inputs. Selection of CO_2as a solvent facilitates the formation of solvent-free extract and successful phase separation in the separator to recirculate the used solvent for reuse. Thus, the operational cost of solvent reduces (less solvent loss) and total energy cost related to solvent collection decreases. This reduced

energy consumption improves the overall sustainability of SCO_2-based extraction processes [Khaw, 2017]. SCO_2E process may not involve any secondary solvent recovery step like MAE or UAE. Thus, solvent disposal cost in this robust technique is zero (for non-polar or slightly polar molecules) or minimum (for recovery of 5% modifier usually applied). It is also noticed that selective separation of targeted biomolecules or active ingredients with their original characteristics is only possible to achieve employing SCO_2E method by tuning pressure and temperature of extraction based on solubility data.

Thereby, SCO_2E method is gaining choice of preference among all these extraction methods in this study to find out its performance in the extraction of natural medicinal plant extracts from two accessible sources, clove buds and turmeric rhizomes, under some modification of extractor bed geometry.

1.5. Role of Essential Oils against Destructive Cell-damaging Free radicals

A critical cause of health disorder is oxidative stress which may lead to degenerative diseases like cancer, dysfunction of the central nervous system, cardiovascular disease, ageing process, immune system decline, etc. [Halliwell, 1992]. Oxidative stress arises when the antioxidant safeguarding system of the human body fails to inhibit the overproduction of cell-damaging reactive oxygen species (ROS) [Sies 1985; Wang 2007]. ROS are generated at a faster rate with multiplicity from either endogenous or exogenous sources and very much aggressive in searching their missing part of electrically unbalanced oxygen atoms from any tissues damaging carbohydrates, proteins, lipids, DNA and RNA [Halliwell, 1991; Pisoschi, 2015; Zhao, 2006]. Hence, the secret to a healthy, youthful, long life is eating more antioxidant-enriched foods and substances to overcome immune deficiency and environmental stress factors [Willcox, 2009].

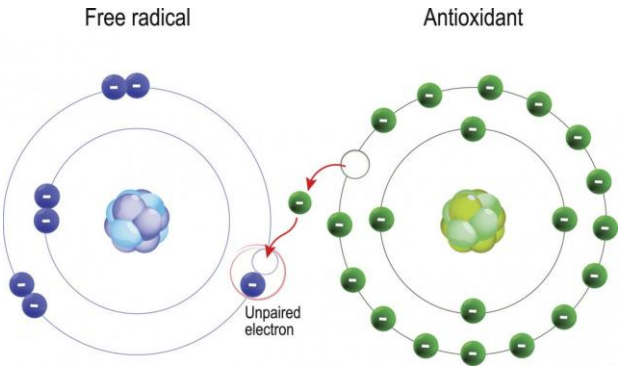

Fig.1.10: Stabilization of Free Radical by Electron Donor Antioxidant Molecule

Phenolic compounds of plants essential oils are excellent antioxidants [Vladimir-Knežević, 2012]. Consumption of these phenolic compounds supplies antioxidants to the human body. Antioxidants donate a free electron to a free radical and stop the free radicals chain reaction. Therefore, these antioxidants play crucial roles in improving antioxidant defense system of the human body, inhibiting the overproduction of cell-damaging reactive oxygen species (ROS), preventing enzymes involved in oxidative stress and binding metal ions responsible for the production of ROS [Dangles, 2012; Sies, 1985; Wang. 2007]. Some well-known antioxidants are carotenoids (such as beta-carotene), vitamin C, vitamin E, lutein, resveratrol, lycopene, and other phytonutrients, etc.

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Chapter 2

LITERATURE REVIEW

2. Literature Review

This chapter provides the background information on supercritical carbon dioxide extraction (SCO_2E) and a review of the available literature relevant to applications of SFE in the isolation of clove oil from its buds and turmeric oil from its rhizomes. This survey was conducted aiming to find out previous SFE optimization studies related to various process parameters that control or affect the performance of the SCO_2E process of clove oil and turmeric oil. Influence of extractor bed geometry was found out as an important factor in the SFE research field. The total search in this chapter has been divided into few subsections as explained below.

The first section presents the necessary information about supercritical fluid extraction and its various aspects associated with the commercial extraction of natural products from different plant resources. Second and third sections provide kinds of literature on clove and turmeric essential oils extraction applying supercritical carbon dioxide extraction process. The second section also covers the reviews on biomedical properties of clove essential oil. The fourth section presents different articles reported on the influence of bed geometry on SFE extraction and correlation developed to couple bed geometry with the process parameters to find out suitable scale-up criteria. However, several available literatures relevant to the present investigation have been presented in the following sections.

2.1 Supercritical carbon dioxide extraction

2.1.1 History of SCO_2 extraction

In 1822, Baron Cagniard de la Tour first time observed the disappearance of two distinct gas and liquid phases of a compressed substance above a particular temperature (critical point) and visualized the presence of a single phase in his cannon barrel experiments [Cagniard de la Tour, (1822) 127, 178]. His discovery was marked as the first record of critical point and supercritical fluid phase. The term "critical point" was first introduced by Dr. Andrews. Fig. 2.1 is a pictorial representation of such disappearance of two distinct gas and liquid phases. From left to right, the temperature is increasing in this picture.

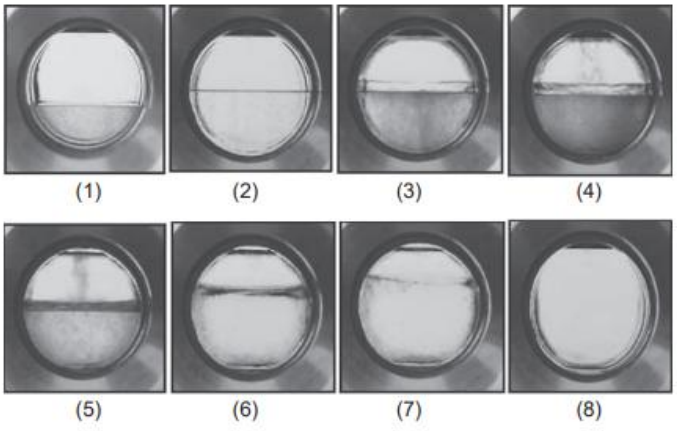

Fig. 2.1: Change from Liquid – Gas Two Definite Phases to One Supercritical Phase
[Source: Otles S. (2008). Handbook of Food Analysis Instruments. CRC Press, pp 29]

In 1879, Hannay and Hogarth found the unique property of solvating power of some solvents above their critical point for solids in their studies on the solubility of solids above critical condition. They noticed that near the critical point, reduced temperature just below the critical temperature of the solvent and sudden reduction of pressure below critical pressure both caused the precipitation of the solid solutes. These solid molecules were detected to re-dissolve easily in the solvents again on raising the temperature by 8^0 to 10^0 or increasing pressure above the critical value. They studied this behavior for various solvents like alcohol, ether, carbon disulphide and tetrachloride, paraffin and olefins and chose different solids like sulfur, chlorides, bromides and iodide of metals, and organic substances like chlorophyll and aniline dyes to study their solubilities. They reported about the pressure-dependent dissolving power of supercritical solvents.

A few years later, Buchner (1906) first got success among a large number of researchers in their attempt to measure the solubility of naphthalene in SCO_2. He also reported about the solubility behaviour of certain nonvolatile organic materials in solvent CO_2. He concluded that their solubilities increased by orders of magnitude above supercritical conditions than expected based on vapor pressure considerations alone.

The first industrial application of supercritical fluid extraction was reported by Zhuse in 1951 [Nautiyal, 2015]. Sovová and Stateva (2011) reported that actual development of SFE as an advanced extraction process was started in the 1960s when advanced analytical techniques detected the presence of trace amount of harmful residual organic solvents in food and pharma products, health consciousness of consumers increased, and people became aware of the non-toxic nature of carbon dioxide which is most popular supercritical solvent having low operation temperature ($T_C = 31.1^0C$) that suits well for thermal labile vegetable substances.

Zosel's various patents in 1964, 1971, 1974 and 1981 and research work on decaffeination of green coffee with SCO_2 added momentum in the advancement of supercritical fluid extraction (SFE) technology. The increasing regulatory attention from the 1970s to eliminate solvent residues from natural products, public awareness for natural products, and enhanced flavor and aroma characteristics of SFE extract resulted in faster growth and large scale operations of SFE from botanical substrates, polymers, and fish oils since 1980s in Germany, the UK, and the US. The reports of Zosel (1978) provided the vital information that the planning for first commercial production of the decaffeination of coffee from coffee beans using SCO_2 was already started in 1978 in Germany. Zosel (1978) suggested three possible ways of the decaffeination process of green coffee beans using compressed CO_2 as a solvent. The first commercial plant of SCO_2E for decaffeination of coffee (capacity 10,000 t/day) was constructed in 1978 and commissioned in 1987 by Hag AG in Bremen [Furusaki, 2001]. Several other commercial plants of SFE to extract mainly food and pharmaceutical products established at that period include hops, tea, red pepper, flavor and fragrances, colorant, tobacco, vegetable oils, and fatty acids. Hubert and Vitzhum in 1978 first reported the extraction studies on the hop, tobacco, and spices using SCO_2. Stahl et al. studied SFE of oils from soybeans, sunflower seeds, and rapeseeds at reasonably low temperatures in 1980. Eisenbach reported about the application of SFE in the fractionation of fish oils in 1984. Since then the SCO_2E techniques has grown enormously for its vast area of applications. Perrut recorded in 2007 the existence of more than 200 industrial plants which were

commissioned in a different capacity for supercritical fluid extraction of a variety of substances all over the world. Some of the largest research fields of applications SCO_2 are the recovery flavours and fragrances, bioactive essential oils, and others which are expanded tremendously.

2.1.2 Properties of Supercritical Fluids

The supercritical region is originated at the critical point due to the formation of only one phase by diminishing the clear interface between liquid and gas phases [C. Cagniard de la Tour, (1822) 127, 178.]. The supercritical fluid region on a phase diagram of a pure substance in a pressure-temperature plot is shown in Figure 2.2.

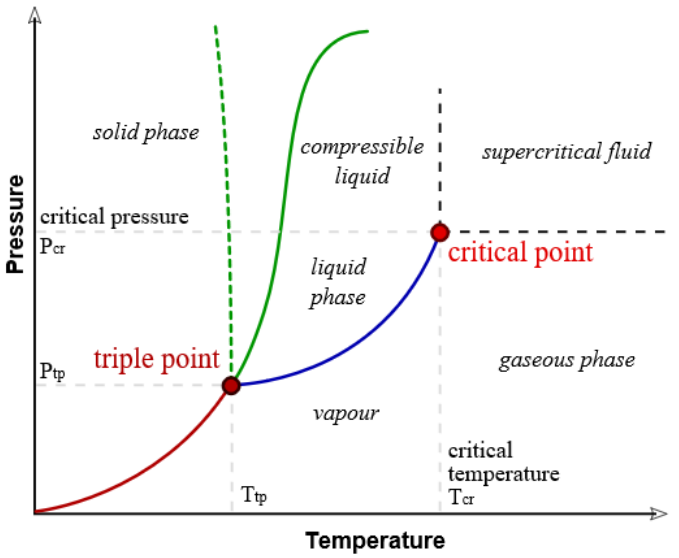

Fig. 2.2: Phase diagram of a Pure Substance showing Different

One important factor related with the design and development of green extraction of natural products mostly from biomass is to use of alternative greener solvents to replace petrochemical solvents due to their negative impact on safety, health and environment [Chemat, 2012]. Selection of alternative solvents should have some essential properties like high solvent power, high diffusivity, low viscosity, and moderate boiling point. They should be biodegradable, non-toxic, non-flammable by nature.

Nowadays, the process of extraction and separation from natural substances using supercritical fluid are preferred worldwide because of the unique solvent characteristics in the supercritical phase which are exceptionally favorable for the recovery of selective molecules from the plant resources. In the supercritical fluid phase, both the gas and liquid essential properties of mass transfer and separation are coupled. These properties are mainly low viscosity, near-zero surface tension, gas-like high diffusivity, and liquid-like sufficiently high density, which add marvelous characteristics to the supercritical fluids to play the role of a novel solvent. These properties influence the mass transfer and separation due to induced mobility, efficient penetration capabilities through the solid matrix, favorable mass transport characteristics, and liquid-like dissolving power with high selectivity, respectively [Williams, 1981]. The comparable physical properties of different fluid states are given in Table-2.1.

The principal advantage of SFE over distillation is the low temperature effectiveness of the supercritical fluids that enhanced their applications to recover heat-labile compounds of low volatility avoiding thermal degradation and decomposition. Another promising characteristic of supercritical fluids is the production of clean extract and extraction residue by the easy recovery of the supercritical solvents; particularly those are gases at the ambient condition like carbon dioxide. The highly tunable solvent density, solvation power, and selectivity with a slight change of process parameters like extraction temperature and pressure in the supercritical region are the unique natures of the supercritical solvents. These unique, finely tuned physico-chemical properties of supercritical solvents are exploited to achieve the selective separation of targeted molecules by controlling extraction pressure and temperature above critical condition. This innovative, clean, and green extraction technology is extremely suitable for the recovery of non-polar substances of medium molecular weight and partially effective to isolate substances with relatively low polarity [Williams, 1981].

Table-2.1: The comparative physical properties of different fluid states [Baiker, 1999]

Physical Property	Gases	Supercritical Fluids (Near critical Point, T_C & P_C)	Liquids
Specific mass (kg/m^3)	0.6 – 2.0	200 – 500	600 - 1600
Dynamic viscosity ($10^3 kg/m.s$)	0.01 – 0.3	0.01 – 0.03	0.2 -3
Kinematic viscosity ($10^6 m^2/s$)	5 - 500	0.02 – 0.1	0.1 – 5
Diffusivity ($10^6 m^2/s$)	10 - 40	0.07	0.0002 – 0.002

2.1.3 Supercritical Fluid extraction Equipments and Instrumentation for Plant Material

Although the SFE technology has multidimensional applications such as extraction from plant materials, nano-particle design, reaction engineering, polymer treatment, waste treatment, etc., still the most extensive commercial application of SFEs contribute in the extraction or recovery of natural products from plant materials using carbon dioxide at its supercritical phase. Like other solid feed processing system, the extraction from botanic material applying SFE technology is carried out in semi-continuous mode. Starting from the first commercial plant of SFE to present laboratory scale, pilot scale and industrial scale units of SFE prefer cylindrical geometry to design the extraction vessel to obtain fixed bed of ground plant matrix [Zosel, 1978; Sovová, 2011]. The lab-scale to industrial-scale SFE units also consists of a high-pressure solvent pump to pressurize and deliver the supercritical solvents at a constant flow rate, a modifier pump particularly for vegetable materials, and one or more separator/s or fractionation chambers to precipitate the recovered substances by depressurisation, reduction of temperature or with the help of some mass-separating agent [Brunner, 1994]. Regeneration and recirculation of the used supercritical solvent are also possible that reduces the operational cost of the industrial units where consumption of supercritical solvent particularly carbon dioxide is very high [Rizvi, 1994]. Multistage separations are often applied when partial fractionation of the natural extract is

required [Brunner, 1987; Reverchon, 1997]. The recovery of various fractions of the extracted compounds can be achieved by implementing stepwise depressurization. Depending on the differential solubility of the soluble fractions in the supercritical fluid, selective compounds can be obtained in a particular separator. Usually, the extraction vessel and the separators are equipped with independent temperature and pressure control system.

2.1.4 Carbon Dioxide as Supercritical Solvent

A wide range of compounds including different organic liquids, noble gases, water, and other inorganic substances can be used as a solvent of SFE. The suitable supercritical fluid for a particular job is selected based on the polarity of the substances to be extracted, operational conditions required to extract them and various safety, health, and environmental factors. Fluids which are environmentally not benign, corrosive, toxic, flammable, and explosive in nature and may cause some health hazards are usually not preferred as supercritical fluids. The critical properties of several common solvents used in SFE are provided in Table-2.2 [Herrero, 2006].

Table-2.2: Critical Properties of some Solvents used in Supercritical Fluid Extraction [Herrero, 2006]

Solvent	Critical Property			
	Temperature (^{0}C)	Pressure (atm)	Density (g/ml)	Solubility parameter δ_{SFC} (cal$^{-1/2}$cm$^{-3/2}$)
Ethene	10.1	50.5	0.200	5.8
Water	101.1	217.6	0.322	13.5
Methanol	-34.4	79.9	0.272	8.9
Carbon dioxide	31.2	72.9	0.470	7.5
Ethane	32.4	48.2	0.200	5.8
Nitrous Oxide	36.7	71.7	0.460	7.2
Sulfur hexafluoride	45.8	37.7	0.730	5.5
n- Butene	-139.9	36.0	0.221	5.2
n- Pentane	-76.5	33.3	0.237	5.1

Depending on the broad area of applications supercritical carbon dioxide (SCO_2) and subcritical or supercritical water are considered as the most promising environmentally benign solvents [Pourmortazavi, 2007; Sovová, 2011]. Subcritical water is an excellent solvent for both polar and non-polar compounds. Main drawbacks of subcritical water are corrosive nature and high operating temperature range (100-374^{0}C) that not suits for temperature-labile substances. The critical temperature of CO_2 (31.1˚C) is far below the critical temperature of subcritical water (374^{0}C). SCO_2 is the most utilized critical fluid due to its moderate critical pressure along with temperature (31.1˚C and 7.38 MPa), the benign effect on the environment, non-toxicity, non-flammability, noncorrosive nature and commercial availability in highly pure state [Rozzi and

Singh, 2002]. Carbon dioxide is GRAS, inexpensive, and less reactive by nature [Phelps, 1996]. Besides, SFE processes employing CO_2 as a solvent can reduce the time of extraction and can be automated [Lehotay, 1995]. Since CO_2 is a gas at normal atmospheric condition, it can produce high purity extract (no solvent residue), and therefore, the costs associated with solvent waste disposal diminishes [Stahl, 1980]. Fig. 2.3 shows the different phases of carbon dioxide.

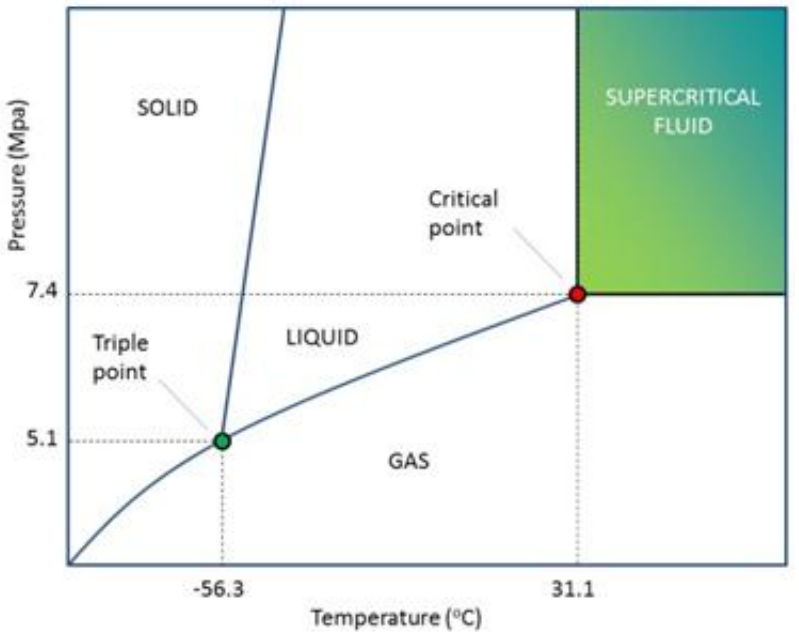

Fig.2.3: Phase Diagram and Pressure-Temperature Critical Values of Pure Carbon Dioxide

In 1861, Tyndal, J. identified CO_2 as a heat-trapping greenhouse gas that causes global warming. Use of CO_2 as solvent of choice for industrial SFE process contributes in the reduction of the atmospheric carbon dioxide level by utilization of a large amount of CO_2 produced as waste gas from its point sources such as cement industries, biomass power plants. Wide range of applications of SCO_2E processes involves high capacity plants in food, pharmaceutical, flavor and fragrance, cosmetic, and beverage industries. Thus extensive development of SCO_2E processes can require a large amount of carbon dioxide to collect from its production points and prevents its release to the atmosphere, which helps to control the global warming sources. In this regard, Moyler (1993) reported in his study that CO_2 obtained as a by-product of the fermentation processes could be used as a source of solvent required for SFE. Thus its use as an important extraction solvent does not increase its level that already exists in the atmosphere. Therefore, the overall adverse effect of CO_2 gas on the earth's ozone layer should not increase from this use of solvent CO_2.

Application of CO_2 as a solvent is limited in case of polar solute due to its zero dipole moment. It shows small polarizability. For the supercritical fluid extraction of non-polar and slightly polar molecules, the choice CO_2 is always preferable. Use of modifiers at lower concentration with carbon dioxide can increase the solubility of the polar solutes in SCO_2 phase. Most common modifiers are methanol, ethanol, water, etc. [Sovová and Stateva, 2011].

Alexandrou et al. (1992) in their study on the recovery of polychlorinated dibenzo-p-dioxins from fly ash achieved higher and complete recovery using supercritical nitrous oxide (N_2O) as compared to SCO_2. Pourmortazavi et al. (2007) also reported that in 1990s, some research works mentioned the choice of N_2O as a solvent for analytical SFE application. The main barrier in its widespread application is its highly explosive nature in samples with high organic content. Therefore, it is not recommended as a solvent of choice for SFE unless it is specifically required.

Moyler (1991, 1993) reported on the quality of yield obtained during extraction using CO_2 and other extraction solvents. It was found that the most natural odor and flavor of extracts were obtained for CO_2 among all available solvents. Grimmett (1981) studied on the extraction utilizing liquid carbon dioxide and observed that "the aromas of extracts obtained by liquid carbon dioxide extraction bear a closer resemblance to the original material than those obtained by organic solvent extraction" [King, 2012].

Nahar and Sarker (2012) reported that SCO_2 is ideal for the recovery of natural substances from botanic materials and is especially recommended when the low temperature of extraction needed for obtaining thermolabile compounds.

2.2 Clove and Its Essential Oil

Clove *(Syzygium aromaticum L.,Caryophillus aromatica L., Eugenia aromatica Kuntze, Eugenia caryophyllata Thunb.* or *E. caryophyllus)* is a valuable and precious spice of high popularity all over the world. It has 23 genera and almost 130 species, many of which are rich in aromatic oil [Rezende, 2013]. The most valuable part of the evergreen tree belonging to the family Myrtaceae is the unopened dried flower buds. This aromatic species is originated from the North Moluccas Islands, popularly known as Spice Island. It is also indigenous in eastern Indonesia, where wild forms are found [Nurdjannah, 2012]. It is widely cultivated in Zanzibar, Pemba, Penang, Madagascar, Caribbean islands, Sri Lanka, southern India and south of China. This medium size (10-20 m), long live (up to 100years) species grow well in the warm, humid climate in the vicinity of the sea [Bhattacharya, 2016]. The leaves of this popular spice plant are also aromatic in nature. At the young stage, unopened immature buds are usually green, and their appropriate harvesting time is identified when they turn to slightly pink at full size. These unopened buds are then dried isolated from any foreign material and graded. Dried clove buds are brownish-black in color. Fig. 2.4 shows different stages of clove buds clearly.

The different useful forms of clove are dried buds, ground clove powder, essential oils produced from dried buds, stem or leaves, and oleoresins. These clove products have a strong fragrance and burning taste. Different forms of clove are mostly useful as folk medicine; food flavouring and preservative agents; pharmaceutical ingredients; and fragrance molecules. In India, it is used in most of the spicy dishes in the form of whole bud or paste or powder, popularly known as "Garam Mashala." Whereas, Indonesia uses half of the cloves produced over the world in making a particular type of clove cigarette (before 2009 popular as "kretek") [Milind, 2011].

Fig.2.4: Different Stages of Clove Buds

The high value and popularity of clove are due to its higher essential oil content and the presence of some major bioactive components (eugenol, eugenyl acetate, and β-caryophyllene) at a higher percentage. Eugenol and eugenol acetate are considered as the most responsible ingredients to control the aroma. Higher eugenol content causes a powerful harsher aroma, while a higher level of eugenol acetate produces a pleasant aroma. For good quality clove higher quantity of essential oil and a greater percentage of major components in oil are preferred.

Nurdjannah et al. (2012) reported the characteristic differences of clove buds at various stages of maturity. It was observed that the harvesting time of clove buds is very much crucial as it influences both the aromatic oil content as well as chemical composition. Immature, fallen, and over-mature or ripe buds will cause quality product (Table-2.3).

Table-2.3: Characteristics of clove buds at several stages of maturity [Source: Nurdjannah, 2012]

Characteristic	**Bud maturity stage**			
	Fallen flower	**Undeveloped bud**	**Fully developed bud**	**Over-ripe bud**
Clove bud				
Water content (% v/b)	12.3	5.0	12.8	6.5
Oil content (% v/b)	13.9	14.9	16.4	16.1
Ash content (%)	4.7	3.8	4.7	6.11
Fibre content (%)	11.8	10.8	8.5	13.3
Si content (%)	0.15	0.11	0.11	0.10
Clove bud oil				
Total eugenol content %[1]	91.0	90.0	94.0	93.0
Eugenol content (%)[2]	54.2	55.6	68.5	72.2
Eugenol acetate content (%)[2]	34.4	36.5	22.0	9.4

[1]Cassia method and [2]By gas chromatography.

Ground clove can be produced by applying milling and/or grinding operation depending on the required level of fine powder. Precautions should be taken to minimize the loss of any valuable volatile oil components from the heat liberated during milling and grinding operation. For large scale production of clove powder or clove oil several techniques such as pre-chilling, water cooling, refrigeration of the grinding chambers and cryogenic grinding at sub-zero temperature have been developed to diminish the adverse thermal effect on the product [Nurdjannah, 2012].

Among 10% essential oil bearing plants from the giant plant kingdom, very few plants species contain high essential oil content (more than 10%). Clove (Syzygium aromaticum) and nutmeg (Myristica fragrans) are two common examples of high aromatic oil bearing plant. Rest other species in this category contain very low quantity barely exceeding 1% of essential oil [Bowles, 2003]. Clove oil is pale yellow in colour with its characteristic aroma and can be recovered extracting buds, leaf or stem of the clove tree. Clove oil consists of several aromatic components with three main bioactive ingredients mentioned earlier eugenol, eugenol acetate, and β-caryophyllene. Their quantities are used as a measure of grading the cloves. Chemical structures of these three active ingredients are given in Fig.2.5.

The published results on chemical compositions of clove oil originated from different plant parts revealed great variability in the chemical compositions. Hossain et al. (2012) identified a total of 26 components from two samples of clove buds originated from India and Indonesia. Jirovetz et al. (2006) reported the presence of 23 compounds in clove leaf oil.

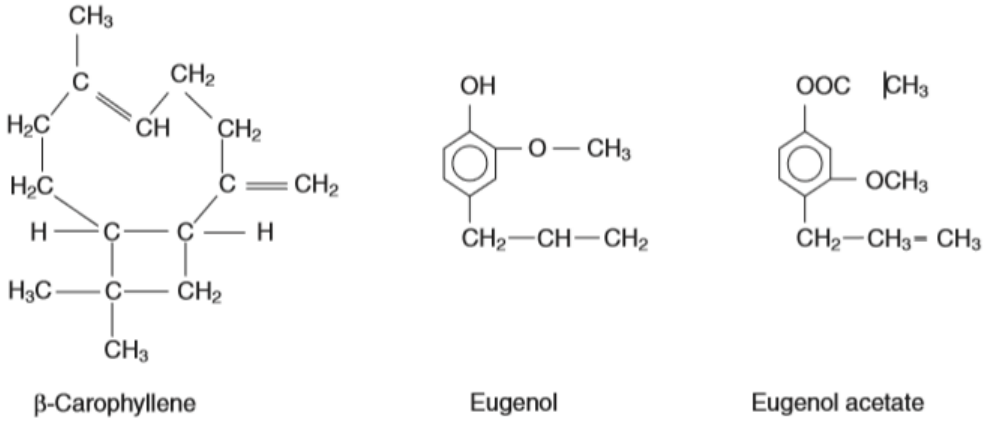

Fig. 2.5: Chemical structures of eugenol, eugenol acetate and β-caryophyllene [Guenther, 1950]

The yield and chemical constituents or quality of clove essential oil are affected by several factors such as origin, variety, maturity, parts of plant extracted, post-harvest processing, milling/grinding technique, method of extraction, extraction operational conditions, post-extraction treatment. Clove buds are enriched with good quality aromatic oil as compared to stem or leaf. Clove buds oil can vary in the range of 15-20% [Gopalakrishnan, 1988]. However, Clove stem and leaf comprise of 5-7% and 3-4% essential oil by weight, respectively.

Nurdjannah et al. (2001) reported an article with information about the characteristic variation of clove oil obtained from buds, stem, and leaf of Indonesian origin (Table-2.4).

Gaylor et al. (2014) aimed to analyze and compare the bud, leaf and stem essential oils of clove sourced from Madagascar, Indonesia and Zanzibar bud, leaf and stem essential oils (Table-2.5).

Table-2.4: Characteristics of clove oil derived from buds, stem, and leaf of Indonesian origin [Nurdjannah, 2001]

Compound	Bud (%)	Range	Stem	Range	Leaf (%)	Range
Essential oil	18	15-20	6	5-10	3-4	1-4
Specific gravity	10425	1004-1057	10495	1048-1056	1030	1030-1060
Refractive index	15296	1528-1538	15320	1534-1538	15295	1520-1540
Optical rotation	-1.1	0-(-1.58)	-1.05	0-(-1.50)	-1.58	-1.58
Total eugenol content	80	85-93	85	89-85	82	78-93
Solubility in ethanol	1:2	1:2	1:2	1:2	1:2	1:2
True eugenol	61.62		80		77.1	
Eugenol acetate	18.72		2.1		trace	
β- Caryophyllene	15.27		12.70		17.02	

Table-2.5: Chemical composition of Clove bud, leaf and stem essential oils [Gaylor, 2014]

RI[a]	Compounds	Bud	Leaf	Stem
1176	Methyl salicylate	0-0.32	tr	0-0.56
1241	Chavicol	0-0.24	0-0.13	0-0.22
1348	Eugenol	72.08-82.36	75.04-83.58	87.52-96.65
1375	α-copaene	0-0.27	0-0.24	0-0.27
1387	Methyleugenol	0 - 0.08	0 - 0.24	0 - 0.15
1420	β-carryophyllene	2.76 - 8.64	11.65 - 19.53	1.66 - 9.72
1453	Iso-cugenol	0-0.24	0-0.24	0-0.83
1465	α-humulene	0.34-1.04	1.38-2.17	0.22-1.31
1494	Eugenyle acetate	8.61-21.32	0-1.45	0.07-2.53
1585	Caryophyllene oxide	0.06-0.37	0.05-0.55	0.14-0.68

[a] Retention Indices on CP Sil 5 Column, tr: trace

Clove is champion among all spices due to its wide range of applications particularly in food, and pharmaceutical industries. Clove active ingredients represent antioxidant, antimicrobial, antibacterial, anodyne, anti-inflammatory, antiviral, antifungal, anti-diabetic, anti-carcinogenic, anesthetic, antiseptic, analgesic nature. In detail, the uses of clove and its products were reported in different literature [Bhowmik, 2012; Milind, 2011; Nurdjannah, 2012]. The global market of clove oil is growing annually at a rate of approximately 5% [Nurdjannah, 2012].

2.2.1 Review on Supercritical Fluid Extraction of Clove

❖ **Solubility of Clove Oil**

The solubility data for the system of SCO_2 and clove buds oil was evaluated by Rodrigues et al. (2002). The solubility was measured considering this aromatic, medicinal, and spice plant's parts and solvent CO_2 consisted of three components: cellulosic structure, extract, and solvent (i.e., pseudo-ternary systems). This basis was based on the consideration of solute-solvent and solute - cellulosic structure strong interactions that can influence the solubility data. The solubility data for clove - SCO_2 system were calculated performing different extraction experiments of clove sample under various operating conditions of pressure, temperature, and SCO_2 flow rate. For a particular extraction pressure and temperature, it is required to identify the appropriate solvent flow rate at which solute-solvent saturation will be attained just before leaving the extractor. The dynamic method used to calculate the solubility was related to the kinetic study of extraction. From the experimental yield vs. time data (obtained using optimum solvent flow rate) overall extraction curves (OEC) were plotted, fitted with two straight lines with the help of spline and solubilities were measured following the dynamic method developed by McHugh et al. (1994). The chemical analysis of the clove oil indicated the presence of eugenol in larger quantity during the constant rate period of extraction for all runs. Therefore, the solubility of the eugenol in SCO_2 was suggested as the principal factor to evaluate the solubility of the pseudo-ternary system. The solubility values for clove buds in SCO_2 were provided in the Table-2.6.

Table -2.6: Solubility measured by the dynamic method for the pseudo-ternary system of Clove- SCO_2

T (K)	P (Bar)	$Y^{*}\times10^{3}$ (kg/kg)	$Q^{*}\times10^{5}$ kg/S
283.15	66.7	258	1.84
288.15	66.0	220	1.74
288.15	66.7	234	1.85
288.15	70.0	230	1.51
288.15	72.0	238	1.65
288.15	80.0	244	1.60
288.15	100.0	277	1.64
298.15	100.0	267	1.65
308.15	100.0	230	1.54

The apparent solubility data of essential oil in solvent SCO_2 contribute appreciably on the development and process economics study of the SFE processes. The apparent solubility of the solutes influences the rate of mass transfer and controls the initial constant extraction rate (CER) period of SFE from the vegetable matrix. The operational cost of a SFE plant strongly depends on the mass transfer rate in CER. Sovová (2012) reported the apparent solubility of volatile oils obtained from various plant parts for solvent CO_2. A SCO_2E experiment was conducted at 400C and 10MPa to calculate the apparent solubility of clove buds, and the slope of OEC was evaluated. The calculated value based on the above mentioned method was reported as 2.3gm clove volatile oil per kg of supercritical carbon dioxide.

❖ **Effect of Extraction Parameters**

Gopalakrishnan et al. (1990) examined the influence of extraction pressure and temperature, period of extraction, and moisture content on the SFE yield and major flavouring components of clove buds. The results of SFE were also compared with the yields and compositions of extracts obtained from hydrodistillation and extraction using liquid CO_2. 100 gm clove feed was extracted in a 200 ml SF extractor module. The pressure effect was studied at 10, 25, 40, and 50MPa keeping extraction temperature and contact time constant at 40^0C and 30 min, respectively. Two temperatures, 20^0C, and 40^0C were selected to study the temperature effect at constant extraction pressure of 25MPa and 30min period. Effect of contact time was verified for 30 min, 60 min, and 120 min extraction periods at 25MPa and 40^0C. For the same operating pressure and temperature, the impact of moisture was studied for two moisture levels $90gm.kg^{-1}$ and $180gm.kg^{-1}$ for 30 min period of contact. 18.1% yield of clove oil resulted from hydrodistillation was almost colourless, free of chlorophyll. Increasing pressure of SFE yielded higher oil (varies significantly from 6.8% to 18.7%) due to increased diffusivity, caused increasing colour intensity from greenish-brown with increasing pressure, raised the percentage of chlorophyll particularly at higher pressure above 25MPa and reduced the content of eugenol acetate and caryophyllene. Significant differences in yields and chemical compositions were observed for extraction studies conducted with SCO_2 (40^0C) and liquid CO_2 (20^0C). Yield (19.3%) obtained with liquid CO_2 was highest compared to only 6.8% yield with SCO_2 and 18.1% in hydrodistillation. Presence of higher molecular weight compounds in clove extract was observed for liquid CO_2. Content of caryophyllene, chlorophyll, and eugenol acetate was higher in hydrodistilled and liquid CO_2 extract as compared to SFE extract. Increasing period of extraction up to 1 hr improved the quantity of yield and eugenol content. Above 1hr extraction period, the yield was not changed remarkably, but the content of caryophyllene, chlorophyll, and eugenol was increased compromising with eugenol acetate part. Increasing moisture also indicated increased yield with a notable rise in chlorophyll (doubled), caryophyllene and eugenol content reducing the eugenol acetate. Low pressure and a short period of extraction were recommended to obtain light colour, sweeter and soft floral odour oil richer with eugenol acetate. Presence of high chlorophyll content was observed as a probable cause of increasing colour intensity of yield. It was concluded that methods of extraction along with other operating parameters contributed significantly on the yields and their quality.

Huston et al. (1991) performed an optimization study on the analytical SFE of Cloves and estimated the effect of SCO_2 densities on the selectivity of various components of milled clove

buds. Extracts recovery was achieved either using solvent recovery or direct on-column interface provided with the GC/MS system. For separation and identification of clove oil components, an ion trap GC/MS system was utilized. The highest yield of 24% clove oil recovered in solvent 2-propanol was achieved from an exhaustive run period of 4.5 hrs and extraction conditions of 400 atm, 40^0C, and 0.959 g/cm^3 SCO_2. For achieving a larger number of extracted components under all extraction conditions, direct interface method was shown more efficient than the solvent recovery method, which caused earlier elution of the solutes. Thus the ability of an on-column interface system was especially worthy for recovery of trace and volatile constituents of complex natural extracts.

Della Porta et al. (1998) also worked on the optimization of clove oil SCO_2E process parameters like pressure, temperature, solvent density to achieve the best extraction and fractionation conditions to obtain pure odor bearing terpene compounds free from any undesired components. This work proposed use of two-stage fractionation to separate byproduct paraffin wax from terpene fraction. SFE experiments were conducted in a 400cm^3 extractor with approximately 150-200gm ground clove buds of 350µm mean diameter. Effects of solvent pressure and temperature on the chemical composition of clove oil were tested in the ranges 8-20MPa and 40-50^0C, respectively. The best extraction conditions were attained at 9MPa and 50^0C with a yield of pure terpene mixed with wax. For separating the wax from terpene fraction, the extract was finally split into two fractions using two separators and applying the knowledge of fractional separation by stage-wise depressurization. The solubility data of wax and clove oil in solvent CO_2 indicated that at around 0^0C the solubility of paraffin wax is near zero, whereas terpenes remain completely miscible in solvent CO_2. 9MPa pressure and -10^0C temperature was successfully applied in the first separator to confirm the precipitation of wax from the clove oil. The second separator was operated at 1.5MPa pressure and 10^0C temperature to allow the release of oil molecules from gaseous CO_2. The highest yield of 20.7% was obtained at optimum operating conditions from an exhaustive run of 630 minutes. Comparatively, low extraction pressure yielded clove oil consisted of a higher percentage of eugenyl acetate (19%) with other two major components eugenol, and caryophyllene in 65.9% and 11.1%, respectively. Presence of higher eugenyl acetate resulted in a sweeter, slightly floral, light yellow and less therapeutic oil as compared to the commercial hydrodistilled oil. This study concluded that extraction pressure above 10MPa and temperature above 50^0C could cause co-extraction of high molecular weight components and other colouring matter together with essential oil.

Yazdani et al. (2005) aimed to find out appropriate SCO_2E conditions of pressure and temperature for selective separation of eugenol and eugenyl acetate from ground clove buds (100µm) size and achieved an optimum operation condition of 353 K temperature and 19MPa pressure at which the number of bioactive components recovered in the extract was reduced to exactly two targeted components. All the SFE experiments of clove buds were conducted to investigate the impact of higher extraction temperature (325 – 416 K) on the quantity and quality of clove oil. Extraction pressure was varied in the range of 11-19MPa. Chemical analysis of the extracts of different runs indicated that with increasing both temperature and pressure within the design range while the yield of eugenol content was increased in the extract, at the same time yield of caryophyllene was dropped. The extracts clove oil of SFE was varied in the range of 12 –

13%. The yield of clove oil and its major components obtained using SCO_2 was compared with the extracts obtained from steam distillation, solvent extraction, and microwave-assisted extraction. The yield of clove essential oil was higher for steam distillation and microwave-assisted extraction. On the contrary, the highest amount of eugenol and eugenyl acetate was obtained from SFE run.

Wenqiang et al. (2007) investigated the influence of three parameters namely, extraction pressure, temperature and particle size of clove buds, on the SFE yield of clove oil and the eugenol content in the extract; optimized these parameters for clove oil extraction using SCO_2 as solvent; and performed quantitative and qualitative analysis of clove oil obtained from SFE runs and three other traditional methods such as solvent extraction, steam distillation, and hydrodistillation. Three levels of each parameter were defined as 30^0C, 40^0C, 50^0C for temperature; 10 MPa, 20 MPa, 30 MPa for pressure and three-degree index for particle size of clove. These factors were investigated with the help of three-level orthogonal array design. Analysis of the clove oil content of SFE runs indicated that the influence of particle size on the yield was crucial within the design range and then temperature and pressure sequentially influence the yield. For selective recovery of the eugenol content of the extract, extraction temperature showed the largest influence. The increase of clove oil content was observed for the rise of temperature from 30-40^0C, an increase of pressure, and a reduction of particle size throughout their design range. Increasing temperature above 40^0C indicated a negligible effect on clove extract and positive impact on eugenol amount. It was demonstrated that increasing temperature above 40^0C caused a significant drop in solvent density that dominated over the positive impact of solute vapour pressure. Increase of solvent density with increasing pressure attributed to the increasing solubility throughout the design domain and resulted as rising of oil yield. It was also noticed that major bioactive compound eugenol was not improved the extract likewise at an elevated pressure above 10MPa due to co-extraction of different high molecular weight fractions. The particle size of the ground clove was inverse with the extracted mass and proportional with the eugenol content. The milling operation of clove sample before extraction helped to rupture the cell boundary and release a larger quantity of oil molecules that became readily accessible by the solvent. Fine particles of clove buds also promoted the co-extraction of high molecular weight molecules. For achieving the highest eugenol content from SFE method, the extraction test was performed at the lowest level of pressure (10MPa) and the highest level of temperature (50^0C). It yielded 19.56% of essential oil with 58.77% eugenol content. Comparison of the results of SFE with that of the other conventional methods indicated that the yield of SFE was higher as compared to that of steam distillation and hydrodistillation, time of extraction was much lower in case of SFE than any of these methods and presence of bioactive antioxidants (eugenol + eugenol acetate) was highest in case of SFE. Therefore, SFE was proposed as the optimum extraction method to produce high quality clove oil.

Zabot et al. (2014) investigated the influence of extractor's bed geometry on extraction kinetics of clove buds oil using SCO_2 and the suitable criteria to obtain similar OECs among extractors with varying geometry. The geometry of the extraction vessel was changed by varying the height to diameter ratio (H_B/D_B) of the extractor bed. Two extractors of same capacity ($1000cm^3$) but varying geometry (H_B/D_B = 7.1, H_B/D_B = 2.7) were designed to find the suitable scale-up criteria for SCO_2E of clove buds. The results of SFE experiments were verified for two

scale-up criteria of constant S/F ratio and equal interstitial velocity of solvent CO_2. Other experimental parameters like temperature, pressure, bed porosity, the mass of feed, and particle size were kept constant. Constant S/F with fixed timed of extraction was established as a suitable scale-up criterion for clove oil SFE process development. It was also noticed that OECs for both geometries closely resembled with each other at a high flow rate of the solvent. For low solvent flow rate, the mass transfer rates for different extractor's geometry were observed to deviate appreciably due to the influence of axial dispersion which was more prominent in the extractor with high H_B/D_B value. Very fine particles can cause a reduction of solvent passage due to dense packing at high pressure and therefore tend to reduce the rate of mass transfer in the extractor with high H_B/D_B. Under the same operating conditions, extraction of clove buds yielded slightly higher oil and upto 9% more eugenol in case of low H_B/D_B. However, larger humulene yield resulted in the extractor with high H_B/D_B. This study concluded that the selection criteria for suitable extractor bed geometry should be based on the selective separation of targeted components and economic feasibility study of the SFE process. THE manufacturing cost of SFE unit is affected remarkably with the reduction of H_B/D_B due to the increase of thickness of the extractor for high-pressure operation.

❖ **Mathematical Modeling and Scale-up Study**

Reverchon et al. (1997) performed SCO_2E experiments on ground clove buds to extract the essential oil at 9MPa operating pressure, 50°C temperature providing two-stage fractional recovery of the extracted mass with the help of two separators operated in series. The operating conditions of both separators were identical with the study of Della Porta et al. (1998) to obtain a yield of essential oil free from paraffin wax. The cumulative yield of clove oil was verified for three different flow rates of the solvent (0.6, 0.9 and 1.2 kg/h) and four different bed heights (from L to L/4, where L indicates bed height of 15 cm). Experimental data were fitted with different mathematical models based on the numerical integration of differential mass balances. The cumulative extraction curves at different solvent flow rates and fluid phase concentration of oil at various bed heights were compared with the respective model curve. The best fit between experimental data and the model curves were obtained using a model with an adjustable parameter accounting the deviation from the equilibrium conditions because of the presence of an external mass transfer resistance.

Martínez et al. (2007) aimed to establish the suitable scale-up criteria for SFE processes and to develop a methodology that can predict the OECs on a large scale independently without any small scale experimental data. Two vegetable material, clove buds, and vetiver were chosen as raw material for this study. The kinetics of SCO_2E of clove buds (particle size of 0.86mm) were determined from SFE experiments conducted at 10MPa and 35^0C using extraction vessels of different scale (5mL and 300mL) and different geometry (varying bed height to diameter ratio) for two scale-up criteria of constant solvent velocity and constant solvent residence time in the extractor. The extraction rate model of Sovová was used to predict large scale OECs from the data obtained from small scale experiment. For achieving this, the mass transfer coefficients of both fluid phase and the solid phase for the small scale experiment were adjusted with the Sovová model and then these data were used to simulate the extraction rate curves for the large scale runs. Finally, experimental, simulated, and model OECs were compared for large scale setup to

evaluate the suitable scale-up criterion. The analysis of the results of two scale-up criteria applied for clove SFE experiments revealed the constant residence time of SCO_2 inside the extractor as the suitable scale-up criterion.

Hatami et al. (2010) aimed to apply the theoretical mathematical model proposed by Goto et al. (1993) for SCO_2E of clove oil and investigated the suitable scale-up criteria between constant velocity and constant residence time from the mathematical modeling results of the scale-up study. The selected model was expressed by two differential mass balance equations of solute (clove oil) in the solvent phase and solid phase based on the desorption–dissolution–diffusion mechanism. Mass transfer equilibrium at the interface of fluid and solid phase was considered. Solving model parameters, the final form of the proposed model was used to compare the model outputs with the experimental data of clove oil obtained from both small scale and large scale SFE units based on scale-up criteria. Both the scale-up criteria were found to fit well with the proposed model of this study for clove –SCO_2 system. This work was also intended to find out the optimum extraction condition of temperature and pressure since the accuracy of optimization ca make any method economically viable. For optimization, a new correlation for clove oil equilibrium constant between solvent and solid phases was developed applying equal fugacity concept between the phases at equilibrium. The optimization technique, genetic algorithm, was employed in this study. The optimal condition to obtain the highest yield of clove extract was evaluated at 10 MPa pressure and 304.2 K temperature.

Since the performance of SFE process depends on several individual parameters as well as their impact of interaction, determination of appropriate scale-up criterion may face complexity. Sometimes a simple criterion fits well as a scale-up method that is possible to adapt easily, would reduce time and huge cost involved with such SFE process development. Prado et al. (2011) performed such a scale-up study on clove buds and sugarcane residue employing a simple scale-up criterion of constant solvent SCO_2 to feed ratio (S/F~3.6) to find the behaviour of pilot-scale setup operated based on laboratory data. The laboratory-scale extractor was of 290 mL capacity whereas; pilot-scale setup was equipped with 5.15 L extraction vessel. Operation conditions of extraction were 313 K and 15 MPa. A 15-fold scale-up of OEC from bench to pilot scale was successfully attained with this simple scale-up criterion. The extract obtained in pilot-scale was 20% higher than that was obtained in laboratory-scale. This criterion can be useful for the industrial scale-up study of SFE.

❖ **Economical evaluation**

Rosa et al. (2005) developed a simple method to calculate the manufacturing cost of the supercritical fluid extraction process for two spice plant materials, clove buds, and ginger rhizomes. The cost was evaluated choosing an industrial extraction unit consisted of two 400L extraction vessel considering 330 days of continuous operation. The estimated manufacturing cost of clove bud essential oil based on this methodology was economically feasible at the chosen operating condition. The lowest manufacturing cost estimated for clove oil was US$9.15/ kg based on 70min period of extraction whereas, the selling price for bulk quantities of clove bud oil was assumed approximately US$40.00/kg based on steam distilled product. Thus, the production cost of essential oil from clove buds applying SCO_2 was obtained appreciably lower than the market price, and SCO_2E was recommended as a feasible process for clove oil extract.

Prado and Meireles (2011) carried out an economic evaluation of supercritical carbon dioxide extraction process to produce clove oil with the help of a commercial simulator SuperPro Designer v6.0. This study was conducted to provide the information required to install an industrial SFE unit of clove oil in Brazil based on economic analysis. The manufacturing cost of clove oil production using SCO_2 was estimated varying the capacity of the extractor or extraction vessel of SFE unit. Three different extractor volumes of $0.005m^3$, $0.05m^3$ and $0.5m^3$ were used for economical analysis. Clove oil SFE experimental data were optimized considering cycle time for each batch, clove oil yield, chemical composition (for quality yield) and cost data. The extract quality was verified upto S/F = 5.11. Simulator results indicated that the manufacturing cost was inversely related to the plant scale. It was lowest for $0.5m^3$ extractor (between US$ 31 and 48 per kg clove oil), highest for $0.005m^3$ extraction vessel (US$ 78 and 126 per kg of yield) and intermediate for $0.05m^3$ extractor (between US$ 39 and 62 per kg of extract). Cost analysis was based on 7920 hr operation time per year, 30 days plant maintenance, the highest raw material cost (US$ 3.51/kg within a one year period). Thus reduction of raw material cost can result in further the reduction of the manufacturing cost of clove oil production. Since the price of SFE yield of clove in the market was around US$ 110/kg, it was concluded that installation of SFE clove oil industry in Brazil was economically feasible with appropriate process optimization and $0.05m^3$ or $0.5m^3$ SFE plants were recommended for installation after cost analysis. The optimized conditions used in this study were 40^0C extraction temperature, 15MPa extraction pressure, 52 minutes time scale for each batch, maintaining a scale-up criterion of constant solvent to feed ratio (S/F=3.65) and CO_2 flow rate of 3.00×10^{-3} kg/s.

2.2.2 Review on Antioxidant and Antimicrobial Activity of Clove Oil

Politeo et al. (2006) investigated the total antioxidant capacities of twelve essential oil bearing spice plants extracts. Essential oils from these plants were isolated by employing the method of hydrodistillation and their extracts were analyzed by the GC-MS. In order to determine the antioxidant ability of essential oils, four different tests were performed: DPPH radical-scavenging effect, FRAP test, antioxidant activity with thiobarbituric acid reactive species (TBARS) and automatic determination of the oxidative stability of fat (RANCIMAT). The highest antioxidant capacity among twelve spice essential oils has resulted in clove oil.

Gülçin (2011) estimated the antioxidant capacity of eugenol, which is a major phenolic component obtained from clove oil. In this study total antioxidant activity, FRAP, CUPRAC assay, DPPH- scavenging activity, ABTS- scavenging activity and DMPD-scavenging activity were evaluated, and results were compared with the antioxidant capacity of Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), a-tocopherol, and Trolox. The results indicated that eugenol had effective antioxidant activity that was comparable with the chosen standard antioxidants capacities in a linoleic acid emulsion system. The reducing power, and DPPH and DMPD radical-scavenging activity of eugenol were found as best among all with highest absorbance indication of high reducing power and low IC_{50} value (for DPPH, 16.06) indication of good scavenging ability.

Ivanovic et al. (2013) determined the first time the antioxidant and antibacterial activities of clove bud extract and clove bud–oregano leaf extract obtained from SCO_2E method. SFE of cloves were carried out at 10 MPa and 40^0C. The highest eugenol content of 64% was recovered

by extracting pure clove buds. Total phenolic content determined from pure clove extract was 530.56 mg GAE/g extract. The antioxidant properties of clove oil were assessed in terms of their free radical scavenging effect (using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals), ferric reducing antioxidant power (FRAP), and peroxide values. DPPH scavenging ability of the tested extracts was expressed as IC_{50} value that was reported as 20.64µg/mL. The antioxidant properties of clove extract determined were comparable with the synthetic antioxidants. Antibacterial activities of bioactive components of clove were tested against selected Staphylococcus, and Enterococcus bacterial strains and results showed moderate antibacterial activities of clove extract. Mixed extract of clove and oregano was observed to enhance both antioxidant and antimicrobial activities.

2.3 Turmeric Rhizome Oil

Turmeric (Curcuma longa L.) is a perennial, tuberous herb belongs to the Zingiberaceae family, native to tropical South Asia. Most important, useful, and valuable parts of this plant are finger-likerhizomes (Fig.2.6), which are practically the underground storage organs of this plant family [Garg, 2002]. Presence of almost 133 species of Curcuma has been reported worldwide [Prasad and Aggarwal, 2011]. Turmeric has ancient origins and evidence says that it was used as a primary spice in the Indian Vedic culture (dates back nearly 4000 years). This spice is bright yellow in colour with strong flavor and higher medicinal value. It is popularly known as "Indian saffron" or "Golden Spice." Now, the aromatic plant is widely cultivated in the tropical countries of India, Indonesia, Sri Lanka, Taiwan, Jamaica, China, Peru, and Haiti [Sinha, 2003]. The turmeric plants grow well at temperatures between 20 - 30°C with a considerable amount of rainfall. The height of the plants usually varies within 1m. India is by far the World's *largest* producer, consumer, and exporter of turmeric and turmeric derived products [Velayudhan, 2012; Sinha, 2003]. India produces more than 90% of the world's turmeric crop, of which 92% is consumed in the domestic sector and the rest 8% is exported per year [Velayudhan, 2012]. Indian turmeric is considered to be the best in the world market because of the presence of active ingredients of potential interest in higher percentage (high curcumin content). The most important trading center for turmeric and place with highest turmeric production rate worldwide is Erode, a city of Tamil Nadu (India). It is popularly known as "Yellow City," or "Turmeric City."

Fig.2.6: Turmeric Plant, Harvested Rhizomes and Dried Rhizomes of Turmeric

The turmeric rhizomes are processed after harvesting to obtain its dried form. The rhizomes are cooked in shallow metallic pans containing 0.05–0.1% alkaline water solution. The rhizomes are

then boiled for 40 minutes to 6 hours depending on the variety. With the help of a wooden comb, the rhizomes are removed and spread on a clean ground for sun drying. The final moisture content of dried rhizomes should be maintained between 8% and 10% (wet basis) [Prasad and Aggarwal, 2011].

The commercial turmeric products like turmeric powder, essential oil, and oleoresin are produced in larger quantities of more than 160,000 tons per year [Furia and Bellanca, 1975; Govindarajan, 1980]. The dark yellow powder product, the most useful form of turmeric for the domestic market, is processed from dried matured rhizomes and is consumed as a daily spice for its natural colour pigments (curcumin), flavour, aroma, and food preservation characteristics by almost one billion populations of the world [Dasgupta, 2014]. Because of several amazing benefits of turmeric, its daily use in a controlled way has high significance for health.

Major industrial consumers of turmeric are foods, pharmaceuticals, confectionery, cosmetics, and textiles [Sinha, 2003; Priyanka, 2018]. This golden spice is used as colouring, flavouring or preserving agent in the manufacturing of various food products such as cheese, butter, yogurt, other dairy products, canned beverages, ice cream, baked products, sauces, yellow cakes, fruit juice, biscuits, sweets, popcorn, cake icings, cereals, and gelatins [Prasad and Aggarwal, 2011]. Medicinal use of turmeric covers broad fields of folk medicine, ayurvedic medicine, and herbal medicine to modern medicine. More than 200 active ingredients were identified from various species of turmeric till date [Gupta, 2017].

These active ingredients are classified into volatile aromatic fractions (or essential oil) and nonvolatile polyphenol curcumin (probably the strongest antioxidant of turmeric) [Dasgupta, 2014]. Turmeric rhizome oil may vary from 3-5% consisting of mainly four different sesquiterpenes (ar-turmerone, α-turmerone, turmerol, and β-turmerone) [Gopalan, 2000; Raina, 2002]. The natural yellow coloured pigments of turmeric are three different curcuminoids of which curcumin [l,7-bis(4 hydroxy-3-methoxyphenyl)-l,6-heptadiene-3,5-dione] is an orange-yellow, crystalline powder and other two demethoxycurcumin and bisdemethoxycurcumin are minor curcuminoids [Sanagi, 1993]. The chemical structures of these major components are given in Fig. 2.7.

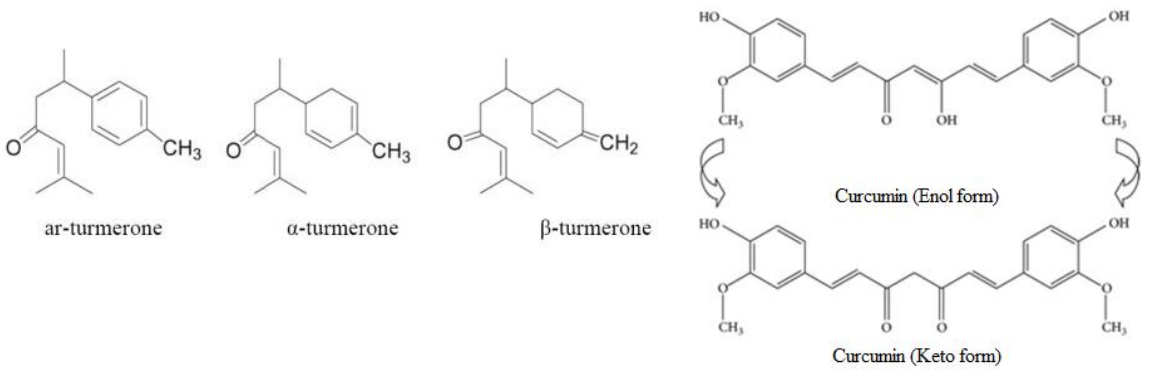

Fig. 2.7: Chemical structures of Turmerones & Curcumin

The studies on biomedical properties of turmeric extract demonstrated that these secondary metabolites possess antioxidant, anti-inflammatory, antifungal, antibacterial, anti-carcinogenic, anti-mutagenic, antiviral, insect repellant, anticoagulant, ant diabetic, antiprotozoal, antivenom, antiulcer, antifibrotic, antifertility, hypotensive and hypocholesterolemic properties [Bagchi, 2012; Priyanka, 2018].

2.3.1 Review on Supercritical Fluid Extraction of Turmeric Rhizomes

Began, G., (2000) studied the influences of three process variables pressure, temperature and solvent SCO_2 flow rate on the SFE and evaluated the optimal values of these three factors to maximize the total oil yield selecting experimental design strategy of Box-Behnken developed under response surface methodology (RSM) of statistical analysis. Three levels of extraction pressure, temperature, and CO_2 flow rates were defined as 10-20-30MPa, 40-50-60^{0}C, and 3-6-9 ml/min, respectively. The graphical plots of yield vs. process variables obtained from RSM analysis explained the influence of extraction pressure, temperature, and solvent flow rate on the extract. Rise of extraction temperature throughout the range of experimental design resulted in the reduction of the extract because of the adverse effect of density reduction of solvent. At low temperature (40^{0}C), increasing solvent flow rate favoured the extraction of turmeric oil upto an operating pressure of 22.5MPa. Increasing solvent flow rate at a constant pressure of 20MPa yielded a larger quantity of oil at any constant temperature. The experimental data were fitted well with the proposed quadratic model equation. The highest yield of 6.65% turmeric oil was obtained at optimum extraction condition when solvent CO_2 was flowing within the level of experimental design.

Began, G., (2000) reported another SFE process parameter optimization study on turmeric oil. This study considered the particle size of turmeric feed as an important factor to control the rate of extraction. Supercritical carbon dioxide extraction runs of ground turmeric was carried out for different level of pressure (20, 30, and 40MPa), temperature (313, 323, and 333K), solvent flow rate [(3.5, 6.7, and 8.6)X10^{-5} kg.s^{-1}] and particle size (0.208, 0.350, 0.589, 0.833, and 1.158 mm). The particle size of solid feed was identified as the most influential factor to control the rate of extraction. The rate and yield of extraction both were inversely related with particle size. Increasing the solvent flow rate resulted in an increase in oil yield. At low temperature (313K) increase of pressure throughout its design range enhanced the rate and yield appreciably by increasing solvent density. Increasing temperature (above 313K) showed a negative impact on the extraction process, which was significant above 30MPa pressure. Selection of suitable extraction temperature and pressure were suggested as crucial factors to attain greater solubility of the solvent. The major identified components of the extract were turmerone and ar-turmerone.

Braga et al. (2003) aimed to determine the quantitative as well as qualitative differences of turmeric extracts derived from turmeric rhizomes employing four different techniques such as SFE, hydrodistillation (HD), low pressure solvent extraction (LPSE), and Soxhlet extraction. The comparative results were prepared based on the extraction yields, chemical composition, and antioxidant activities of the turmeric extracts. The SFE was carried out using supercritical carbon dioxide and two co-solvents in different proportions. Solvents and co-solvents used in the extraction process were ethanol, isopropyl alcohol. Co-solvents improved the recovery of curcuminoids, which are less soluble in SCO_2. The co-solvent effects were verified using solvents

in their pure forms as well as their mixture in equal proportions. Chromatographic Analysis was performed to determine most of the components except curcuminoids, which was quantified using UV-spectrophotometer. The highest turmeric yield of 27% by weight resulted from Soxhlet extraction for a solid-solvent ratio of 1:100. The lowest amount (2.1%) of turmeric oil yielded in the hydrodistillation process. SFE yields varied in the range of 5.7-8% with the highest yield detected in case of solvent mixtures of ethanol and isopropyl alcohol. Chemical analysis of extracts obtained by various techniques indicated more than sixty percent of the extract consisted of ar-turmerone, (Z)-γ-atlantone, and (E)-γ-atlantone, except the extract of Soxhlet extraction. The maximum fraction of the yield (76.5%) was identified containing only two components ar-turmerone, and (Z) -R-atlantone. The important natural die pigment, curcuminoids, was recovered at the highest quantity (8.43%) in Soxhlet extraction using the solvent mixture. Powerful antioxidant activities were demonstrated in the yields of Soxhlet and LPSE. From these results, the soxhlet extraction method was not recommended despite the detection of the highest yield due to loss of major bioactive components. Among LPSE and SFE methods, SFE was advantageous due to the use of very less quantity of organic solvents, recovery of which was related to the reasonable cost of the LPSE process.

The powerful bioactive component and main natural pigment present in turmeric products is curcumin [l,7-bis(4-hydroxy-3-methoxyphenyl)-l,6-heptadiene-3,5-dione], whereas main chemical constituents of aromatic oil of turmeric rhizomes are sesquiterpenes turmerone and ar-turmerone. The chemical analysis of SFE extracts using supercritical fluid chromatography (SFC), which was coupled on-line with SFE unit, was established as a successful technique because of the similarity in their instrumentation and parameters. The SFE coupled with SFC for extraction of turmeric rhizomes and quantification of turmeric extracts, respectively, was performed by Sanagi et al. (1993). The study was conducted in several stages. Extraction and analysis of turmeric and its extract were performed using two solvents (petroleum ether and methanol), pure carbon dioxide (at 60^0C, 25MPa and a flow-rate of 2mL/min), and carbon dioxide modified with methanol (10-30%). During solvent extraction, the petroleum ether was added before methanol to extract particularly nonpolar components. The results of GC analysis depicted a similarity in the chemical composition of the extract obtained by pure carbon dioxide and petroleum ether. The success of SCO_2E technique for removing low-polarity substances was thus established. The preference of SFE technique was because of its easy solvent separation stage. The optimum condition of SCO_2E-modifier system in the recovery of curcumin was detected at 60^0C temperature and, 25MPa solvent pressure using 20% methanol modified SCO_2 (2.0 mL/min). The recovery of curcumin in this technique was greater than 90%. The SFC separation condition of curcumin was optimized at 60°C and 25MPa, using 25% methanol modified CO_2. The chemical analysis result of on-line coupling SFE-SFC system to separate curcuminoids was comparable with that of the high-performance liquid chromatography (HPLC).

Chang et al. (2006) aimed to quantify three turmerones in purer form from the turmeric extract obtained by SCO_2E. This study was also conducted to optimize the SFE process conditions to maximize the turmerones extract by applying two parameters RSM (Pressure and temperature). Four process parameters such as extraction pressure (5.9-34.1MPa), temperature (300-356K), solvent flow rate (5.2-20.8 g/min), and particle size (0.177, 0.420, 0.840 mm) were selected in this study. Investigation on the effect of particle size revealed that larger, as well as

very fine particles, could not favour the rate of extraction. There was an optimum particle size of 0.42mm that produced the highest yield and larger quantity of three turmerones. A prolonged period of extraction above 252min was identified to induce decomposition of turmerones. Results of RSM reported that the yield of turmeric oil was varied from 0 to 6.47 wt%, three turmerones content varied from 0 to 40.50 mg/g turmeric, and purity of three turmerones in the extract ranged from 0 to 71 wt%, respectively. The optimum extraction condition of 320 K and 26 MPa were identified from RSM analysis to extract turmeric oil containing 71 wt% purer turmerones efficiently. Increasing pressure up to 26MPa demonstrated significant improvements in oil yield, three turmerones content, and the purity of the turmerones. Extraction temperature affected the yield negatively. In the purification study, 86 wt% pure Ar-turmerone and 81 wt% pure α+β-turmerone were separated satisfactorily by employing a two-step liquid-solid chromatography method.

Carvalho et al. (2015) reported on the techno-economic evaluation of turmeric oil and extraction of its bioactive component ar-turmerone. This work was intended to find out the influences of extraction temperature, pressure, and operation time on the yield of essential oil and ar-turmerone content and determine the manufacturing cost. Dried ground turmeric powder was extracted in a laboratory-scale SFE module using SCO_2 flowing at 8.4×10-3 kg/min. The experimental design of this optimization study of SFE process was based on full factorial design consisted of three levels of temperature (313, 323 and 333K) and six levels of pressure (10, 15, 20, 25, 30 and 35 MPa). A constant solvent (S) to solid feed (F) mass ratio (S/F) was maintained constant at 12.1. The kinetic study of SFE was carried out at some selective extraction conditions (313 K-20 MPa, 333 K-20 MPa, and 333 K-25 MPa) decided from the results of previous assays. Chemical analysis of extracts was performed in a gas chromatography (GC-FID) instrument. The economic analysis of the SFE process was carried out in the commercial simulator Super Pro Designer 8.5®. The extraction yield of turmeric oil and ar-turmerone content were observed to vary in the same way with variation in extraction pressure and temperature. The contradicting influence of density reduction of solvent and solute vapour pressure rise with the increase of solvent temperature was identified as predominant over 20MPa pressure. The highest yields of turmeric oil and ar-turmerone (6.4% and 1.14% of the extract, respectively) were obtained at 333 K and 25 MPa operation conditions. Total numbers of 30 compounds were detected in the different extracts. Three turmerones (ar-, α- and β-turmerone) were present in high concentrations (≈ 75%) in the extracts. At optimum extraction condition, the manufacturing cost (COM = US$ 178.8/kg extract) for a SFE unit containing two extractors of capacity 0.005-m^3 was estimated as lowest.

Priyanka et al. (2018) investigated the effects of five operating parameters and their interactive effects on the extraction of essential oil from turmeric rhizome using SCO_2. The selected parameters were extraction pressure, temperature, solvent flow rate, particle size and presence of co-solvent (ethanol) that were varied in the ranges from 20-40 MPa, 40-60^{0}C, 5-15 g/min, 0-0.8 mm and 0-15 % of solvent flow rate, respectively. This study was aimed to find out the optimal conditions of these five parameters to maximize yield, turmerone content, and curcumin content. The experimental runs were designed using Central composite design (CCD) developed under the Response Surface Methodology (RSM). The yield of turmeric was varied from 2 to 5.3 wt%. Turmerone and curcumin were identified as two principle compounds of the

extract. Impact of extraction temperature and pressure within this design range were slightly positive on the yield, whereas, solvent flow rate affected moderately the yield of turmeric. Influence of particle size and presence of co-solvent were the most influential factors to control the yield. Larger as well as very fine particles were identified as unfavorable to increase the yield. A moderate particle size (0.45mm) was recommended in this study to maximize the extract. The presence of co-solvent was attributed to the significant improvement in the extract because of the increasing polarity of the solvent. Curcumin isolation was found to influence remarkably by addition of co-solvent rather than any other operating parameters. Most of the operation parameters, except pressure, showed some complex behavior on total turmerone content. Larger particles and the presence of co-solvent both caused reduction of turmerone fraction in the yield. The increasing intra-particle mass transfer resistance in larger particles and moderate solubility of turmerone in ethanol were explained as the probable causes of yield reduction. The yield of turmeric was initially found to reduce with increasing temperature from 40-50^{0}C, after which further increasing trend was noticed. The adverse effect of density drop with increasing temperature dominated over the positive influence of vapour pressure in the range 40-50^{0}C. Increasing solvent flow rate provided a favorable effect on yield upto a certain value of 10gm/min. At very high solvent flow rate, insufficient time of contact between solvent and turmerone resulted as a drop in turmerone content. Experimental data were fitted well with a quadratic model based on statistical analysis. Finally, an economic assessment of turmeric oil production at an industrial scale using SFE method was executed.

Chassagnez-Méndezl et al. (2000) examined the influence of raw feed drying temperature and addition of ethanol modifier with SCO_2 on the amount and quality of extract obtained from turmeric rhizome. Curcuminoids content was selected to study the quality of the product. The cleaned turmeric rhizomes were dried in an oven under the condition of air circulation at two different temperatures at 343 and 378 K for 24 h. The extraction experiments were carried out using pure SCO_2 solvent and ethanol modified SCO_2 solvent. The operation pressures of extraction were 25 and 30MPa. The chemical composition of turmeric oil was evaluated using gas chromatographic-mass spectrometry instrument. The curcuminoids content of the oil and the residual solid were determined using UV-spectrophotometer and HPLC. The presence of curcuminoids content in the extract was higher for the low temperature of drying and extraction using ethanol modifier with carbon dioxide solvent. High temperature drying attributed to the significant loss of oleoresin consisting of curcuminoids pigments by volatilization. Effect of high pressure also observed to favour the extraction of curcuminoids and total oleoresin at a constant temperature of 318K. Addition of modifier presented a crucial role in increasing the total yield and minimizing CO_2 consumption. Because of the extraction of several other polar components along with curcuminoids, complete extraction of curcuminoids using modifier was not achieved in this study. At the same time, separation of the modifier from the extract was included as an additional step of the total process. The kinetics of SCO_2E of turmeric rhizomes was also studied. A desorption mass transfer model described by Tan and Liou was applied to describe the solid phase mass transfer, and a convective mass-transfer model was used to describe fluid phase mass transfer. It was observed that the experimental data were fitted well with the proposed mathematical model of this study.

2.4 Review on Bed Geometry of SCO_2 Extraction Vessel

Meireles, M.A.A. (2008) recommended that extraction vessels of at least 50 cm^3 capacities should be used to perform the kinetic study of SFE of natural plant materials. The extraction vessels should be designed with this minimum volume to obtain complete OECs. Thus, any lab-scale study using vessels with lower than this capacity may not be useful for scale-up study. For very small vessel, the loss of extract within the tubing was observed reasonable compared to the total extract.

Both groups of Carvalho et al. (2005) and Moura et al. (2005) aimed to determine the effect of geometry of the extractor on the SFE kinetics of rosemary (Rosmarinus officinalis) and fennel (Foeniculum vulgare), respectively, and developed two correlations between bed geometry and process parameters that were allowed to predict the required operating condition to attain specific OECs. Combining the geometric parameters (height and diameter of the extractor) with some process parameters for two different extractor units, SFE I and SFE II, two empirical relationships were proposed:

$$\frac{Q_{2CO_2}}{Q_{1CO_2}} = \left(\frac{F_2}{F_1}\right)^2 \times . \left(\frac{H_{b1}}{H_{b2}}\right) . \times \left(\frac{D_{b1}}{D_{b2}}\right) ------(2.1)$$

$$\frac{Q_{2CO_2}}{Q_{1CO_2}} = \left(\frac{F_2}{F_1}\right)^2 \times . \left(\frac{H_{b1}}{H_{b2}}\right) \times . \left(\frac{D_{b1}}{D_{b2}}\right)^3 ------(2.2)$$

Where, Q_{CO2} indicated the mass flow rate of CO_2 (kg/s), F was the mass of feed (kg), H_b, was the extractor bed height (cm) and D_b was extractor bed diameter. Eq. (1) represented a useful relationship to predict the total amount of extractable solute (X_0) from different SFE units by calculating the solvent flow rate for a given feed mass and bed geometry (Hb and Db). Similarly, Eq. (2) was useful to maintain or reproduce the same kinetic behavior in two different SFE units, for a given feed mass and bed geometry by determining the required solvent flow rate.

Sánchez-Vicente et al. (2009) investigated the effects of extraction cell size and solvent flow rate on the SFE of peach seed oil. Two different tubular extraction cells of capacity 230 cm^3 (cell A) and 16.7 cm^3 (cell B) were used in this study. The same operational conditions of pressure (15-19.8 MPa), temperature (313-324K), and particle size (0.25-0.35 mm) of the feed were maintained in both extractors. The higher flow rate of ethanol modified SCO_2 was employed in cell-B as compared to cell-A. Feed sample in cell-A was externally mixed with glass balls before charging inside extractor cell, while, cell-B was extracted at the wholly filled condition. The obtained yields from both set-ups were observed to vary slightly. The yield of cell-A was greater with negligible differences in OECs. For a given constant flow rate of solvent applied for both cells, the higher superficial velocity and smaller residence time of the solvent in cell-B (smaller one) resulted in the reduction of yield. However, the yield of cell-B should not drop significantly because of the predominant solubility control mechanism over convective transfer in the initial stage of extraction.

Zabot et al. (2012) reported on the role of different process parameters affecting the rate and yield of SFE of natural bioactive components from plant materials. In this study, the

emphasis was given on the influence of the bed geometry of the extraction vessel on the performance of the extraction process. The results were indicated that the geometry of the extraction vessel influenced the shape of the OECs because of its influence on the extraction rate. The study on the geometry of the extractor was identified as more crucial during the scale-up study. The scale-up studies reviewed in this search revealed that the successful scale-up of a SFE process was possible when similar OECs were obtained for SFE units with different extractor bed geometries. To attain this condition some correlations among process parameters were found to be maintained, such as the ratio of extractor height to diameter (H/D), the ratio of solvent mass to botanic feed mass (S/F), etc. The H/D ratio was observed very much crucial as it influenced both the extraction performance and economy of the process. The increase in the diameter of the extractor attributed to the increase of the thickness of the vessel that caused the increase of installation cost. This investigation found that a wide range of extractor capacity (2 cm^3 to $2x10^4$ cm^3) and H/D ratios (1.9 to 63.6) were applied in SFE processes for a variety type of materials. Different H/D ratios were found to fit the extraction of different materials. Usually, increasing H/D ratio effects on extraction were explained in terms of increasing mass transfer resistance and back-mixing of the solvent. Extractor with smaller H/D ratios was lead to the heterogeneity in the distribution of solvent.

Zabot et al. (2014) evaluated the influence of two different bed geometries of two same capacity extraction vessels on the OECs of rosemary extract obtained by SFE. The geometry of the extraction vessel was defined in terms of height (H_B) to diameter (D_B) ratios of the vessels (E-1: H_B/D_B = 7.1; E-2: : H_B/D_B = 2.7). A scale-up criterion of fixed solvent mass to feed mass (S/F) ratio for both beds was also investigated. All other major process parameters that can influence the extraction rate were maintained constant. The Kinetic parameters were determined from experimental OECs by fitting three straight lines using spline. The nature of OECs and the results of the kinetic study revealed that extractor bed with small height to diameter ratio was preferable to recover the rosemary yield. The recovery of major components of rosemary was also identified to vary with bed geometry. Variation in the yield and quality of extract in different geometries revealed that proposed scale-up criterion was unsatisfactory for rosemary extraction. This study also listed out some other SFE study conducted to establish suitable scale-up criteria for several other plant-based materials (Table-2.7).

Table.-2.7: Important aspects about the criteria used for geometry shift and scale up in SFE processes.

Raw material	Criterion	Remarks about the criterion	Refs.
Ginger (Zingiber officinale)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Suitable: the OECs were similar when a 17-fold scale up was developed	Prado, 2010
Fennel (Foeniculum vulgare)	$\frac{Q_2}{Q_1}=\left(\frac{F_2}{F_1}\right)^2\times\frac{H_{B1}}{H_{B2}}\times\frac{D_{B1}}{D_{B2}}$	Suitable: the proposed empirical model seemed to be proper for obtaining fennel extract in different bed geometries	Moura, 2005
Sugarcane bagasse (Saccharum spp.)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Not suitable: the kinetic yield for obtaining octacosanol-rich extracts was larger in H_B/D_B = 5.9 than in H_B/D_B = 2.3	Prado, 2010
Grape (Vitis vinifera)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Suitable: similar shapes of the OEC revealed that the criterion was successful in reproducing kinetics behaviors in different scales	Prado, 2012
Clove (Eugenia caryophyllus)	$\frac{Q_2}{A_2}=\frac{Q_1}{A_1}$	Not suitable: maintaining the solvent velocity constant has not been indicated by the authors, because it affects the mass flow rate	Martinez, 2007
Clove (Eugenia caryophyllus)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Suitable: maintaining an equal S/F ratio and an equal time of extraction for using different bed geometries proportionated achieving similar kinetic parameters, mainly the parameter M_{CER}	Zabot, 2013
Lemon verbena (Aloysia triphylla)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Not suitable: the criterion was not valid by comparing the yields in different geometries: H_{B1}/DB1 = 2.3 and H_{B2}/D_{B2} = 5.0	Prado, 2010
Peach (Prunus persica)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Not suitable: yields of different magnitude have been reported	Sánchez-Vicente, 2009
Black Sage (Cordia verbenacea)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Suitable: the OEC for several bed geometries were overlaid	Quispe-Condori, 2008
Pepper (Capsicum frutescens)	$\frac{Q_2}{F_2}=\frac{Q_1}{F_1}$	Not suitable: clear differences for the convective mass transfer coefficient (k_{YA}), between laboratorial and pilot scales, were cited	Silva,2013
Macela (Achyrocline satureioides)	$\frac{Q_2}{Q_1}=\left(\frac{F_2}{F_1}\right)^2\times\frac{H_{B1}}{H_{B2}}\times\frac{D_{B1}}{D_{B2}}$	Not suitable: differences in the kinetic parameters between the bed geometries were presented	Takeuchi, 2009
Annatto (Bixa orellana L.)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Suitable: similar kinetic yields were attained in both beds (H_{B1}/D_{B1} = 2.3; V_1 = 0.3 L; H_{B2}/D_{B2} = 6.0 and V_2 = 5 L)	Albuquerque, 2013
Rosemary (Rosmarinus officinalis)	$\frac{Q_2}{Q_1}=\left(\frac{F_2}{F_1}\right)^2\times\frac{H_{B1}}{H_{B2}}\times\left(\frac{D_{B1}}{D_{B2}}\right)^3$	Not suitable: differences in the extraction curves for the tested H_B/D_B ratios were reported	Carvalho, 2005
Rosemary (Rosmarinus officinalis)	$\frac{S_2}{F_2}=\frac{S_1}{F_1}$; t_E=Constant	Not suitable: different behaviors of the OEC and different profiles of extract composition for each bed were observed	Zabot, 2014

S, mass of solvent; F, mass of raw material; t_E, time of extraction; Q, solvent mass flow rate; H_B, bed height; D_B, bed diameter; V, bed volume; Refs.: reference.

2.5 Conclusion

Based on the survey of the literature as described above it was noticed that despite the strong influence of the geometry of the extractor bed on the process of extraction of active ingredients from herbal resources, studies on the geometry of the extraction vessel of SFE module were directed with a consideration of conventional cylindrical shape with varying bed height to bed diameter ratio and expanded capacity from lab scale to pilot scale or industrial scale. For the reduction of the mass transfer resistance within the pressurized packed bed extraction vessel loaded with ground botanical mass, application of any other geometry other than common type cylindrical geometry was not found in the survey. Thereby, this research work has been conducted to investigate the bed geometry influence on the SFE performance by designing an extractor with unique geometric modification.

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Chapter 3

AIMS AND OBJECTIVES

3. Aims and Objectives

From the preceding discussion, it is established that SF based technology uses a promising green extraction way towards diminishing the use of hydrocarbon solvents using an alternative green solvent, SCO_2 having no negative impact on health, safety, and environment. SCO_2E can extract plant-based bioactive molecules selectively in a more concentrated natural and purer form which have multifunctional activities to improve the living standards of modern civilization. Primary applications include use of natural essential oils extracted from plants in food products, natural medicines, and aroma products to promote healthy oxidative stress free life or improve body immunity or treat diseases.

The successful extraction by this progressive method depends on various parameters such as temperature, pressure, particle size, solvent flow rate, the maturity of biomass, time of extraction, use of co-solvent, level of moisture in the feed, porosity of feed bed, bed geometry of extractorand cost of extraction.

The extraction unit or the extractor is the heart of the SFE system. Geometrical parameters of extractor have a significant influence on the overall extraction curve (OEC), extraction kinetics, and scale-up of SFE processes.The reduction of operational cost of SFE process is highly related to the time of extraction that is possible to decrease by proving an environment that increases the rate of SFE of important bioactive components. The present work is aimed to design a particular type solid bed extractor towards the reduction of overall extraction period by improving the rate of extraction to enhance the process economy without compromising with the quality of products from various plant matrices. To exercise the above tasks, the main objectives of present works are –

i) To develop the extractor for SCO_2E a unique concept of annulus bed geometry is introduced first time to the best of my knowledge by modifying the geometry of conventional cylindrical extractor.

ii) To compare the performance of both types of the annulus and cylindrical extractors for SFE of aromatic plants.

iii) To optimize some important operating parameters of SFE including bed geometry under the consideration of experimental design.

iv) To establish the overall extraction curves for performing kinetic studies and developing the mathematical model equation.

v) To determine and compare the quality of yield obtained from SFE using standard testing methodology.

vi) To determine the antioxidant properties of the natural extract based on standard methods.

vii) To make recommendations on extractor design to maximize the recovery of essential oils using SFE.

In order to achieve the first objective of the present study, three solid extractors of two geometric configurations will be developed. These extractors will be used to load the plant materials externally before transferring them to the central extraction vessel of same dimensions built in the main SFE module. One extractor will be cylindrical in geometry that type is used conventionally. Other two extractors will be annulus in geometry with varying annular diameter keeping external diameter and height same as first type cylindrical geometry.

Once the extractors will be ready to start the SFE of plant materials following investigations will be carried out to compare their performance efficiency:

a. Two different essential oil bearing plants raw materials will be used separately as feed. Their choice would be based on their level of oil content (higher/lower side) to perform comprehensive experimental studies in these extractors to examine their proficiency to extract oils–

   i. Matured fresh clove buds (*Syzygium aromaticum*) will be used as raw feed sample considering its high aromatic oil content (>10%), ease separation from its source applying moderate pressure and temperature (reported 40^0C and 15MPa by Prado et. al., 2011) by SCO_2E compared to many more plant parts (suffering from high-temperature, high-pressure operations).

   ii. Matured dried turmeric rhizomes (*Curcuma longa L.*) will be used as another raw material considering its comparatively low aromatic oil content (<10%) and major commercial role of India in producing turmeric products [Sikkhamondhol, 2009; Sinha, 2003].

b. SFE experiments will be designed giving priority to modified extractor bed geometry as significant extraction rate controlling factor (along with other operating parameters like temperature, pressure, particle size) to evaluate its roleon extracting essential oils.

c. SCO_2E process parameters including bed geometry will be optimized to maximize the yield of essential oils of the selected raw materials applying the techniques of rigorous analysis of process parameters and outcome of the process (i.e. yield) under statistical analysis. It is expected that this study will be helpful to determine the optimum operational conditions under design considerations and explain the role of individual factor as well as 2-factor interactions to improve the yield.

d. Important kinetic parameters of essential oil extraction for varying bed geometry will be determined from OECs by linear fitting to compare the rate of extraction and efficiency of particular bed geometry.

e. Before recommending the favourable bed geometry for essential oil extraction using SCO_2 as a solvent, it is also important –

i. To perform the quantitative as well as qualitative analysis of the oil extracts obtained from various runs to establish that the modification of bed geometry will not compromise with the quantity as well as the quality of essential oils in terms of their bioactive molecules. The quality of extract will be checked using standard methods of detection with the help of an advanced standard gas chromatograph-mass spectrometer and its solution software.

ii. To evaluate different antioxidant assays using standard methods such as DPPH assay, to find out how much this essential oil will be capable of delaying or inhibiting the destructive oxidation processes that are related with the defence mechanism of human beings.

References:

Sikkhamondhol, C., Teanpook, C., Boonbumrung, S., Chittrepol, S., (2009). Quality of bread with added turmeric (Curcuma longa): powder, essential oil and extracted residues. Asian journal of food and agro-industry, 2(4): 690–701. http://www.ajofai.info/Abstract/Quali...

Sinha, K.K., 2003. Spices and Flavoring (Flavouring) Crops / Tubers and Roots. In: Caballero, B., Finglas, P., Toldra. F. (ed), Encyclopedia of Food Sciences and Nutrition, Second Edition, Academic Press,pp 5486-5491.

Prado, J.M., Prado, G.H.C., and Meireles, M.A.A., (2011). Scale-up study of supercritical fluid extraction process for clove and sugarcane residue. The Journal of Supercritical Fluids 56, 231-237, doi: 10.1016/j.supflu.2010.10.036.

Chapter 4

MATERIALS AND METHODS

4. Materials and Methods

To execute the objectives elaborated in the previous chapter to accomplish the aims identified based on a thorough literature search in Chapter 2 the present study was carried out.

This chapter presents the details of various raw materials and chemicals used for experiments and analysis,details of the experimental setups and different instruments used in the present study, and the explicit descriptions of experimental procedures used to generate all the datato investigate the statement of the problem.

4.1 Experimental Materials

4.1.1 Raw materials used for Extraction

i. Buds of Clove - Quality flower buds of clove as available in the local market were purchased from Haldia (West Bengal, India).

ii. Turmeric rhizomes - Matured dried turmeric rhizomes were also procured from the local market of Haldia (West Bengal, India).

4.1.2 Chemicals used in the Extraction of Plant Material

i. Carbon dioxide having purity 99.99% used in the extraction experiments was supplied by a local supplier, Bharat Oxytech Pvt. Ltd., Haldia (West Bengal, India).

ii. GR grade n-hexane (Boiling point: 69^{o}C) used in the Soxhlet Apparatus for solvent extraction of essential oils, wasobtained from Merck.

4.1.3 Chemicals used for Chemical Analysis of Essential Oils

i. Acetone

ii. Helium

4.1.4 Chemicals used for Bioefficasy Testing of Essential Oils

Chemicals used in this study are listed below-

i. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

ii. Ethanol

iii. Ferric chloride ($FeCl_3$,$6H_2O$)

iv. Ferrous chloride

v. Ferrozine

vi. Folin-Ciocalteu reagent

vii. Gallic acid

viii. Phosphate buffer

ix. Potassium ferricyanide

x. Sodium bicarbonate

xi. Trichloroacetic acid, etc.

All reagents used were of GR grade and products of Sigma Chemical Co. (St. Louis, USA) or BDH Chemical Ltd, Poole, UK.

4.2 Main Extraction Equipments:

i. Supercritical Carbon Dioxide Extraction Unit

ii. Soxhlet Apparatus

4.3 Analytical Instruments :

i. Moisture Analyzer (SARTORIUS MA45C)

ii. Gas chromatograph-mass spectrometer (GC-MS),GCMS-QP2010 SE (SHIMADZU, Kyoto, Japan)

iii. UV-Spectrophotometer (Shimadzu UV-vis 1800Spectrophotometer)

4.4 List of Instruments/Equipments used for Experiment or Measurement:

i. Horizontal tray drying unit having air evacuated system (Supplier: M/S Instrumentation India, Kolkata)

ii. Mixture grinder (Philips Mixer Grinder HL7720)

iii. Sieve Shaker & Sieves

iv. Precision balance (Citizen CTG 602)

v. Digital Lab Balance (Testing Instruments Mfg. Co. Pvt. Ltd)

vi. Centrifuge

4.5 Supercritical Carbon Dioxide Extraction Unit

Extraction operations of aromatic plant materials were experimentally performed using a semi-continuous flow type SFE module (Model No: CSL/SCF/1L2/400) procured from M/s Chemtron Science Laboratories Pvt. Ltd., Navi Mumbai, India.

The main components equipped in this module include-

i. High-pressure Liquid CO_2Pump - HP DOSING PUMP MODEL# UMBL-30: PLUNGER
- Maximum Delivery Pressure 50MPa
- Drive: Mechanical
- Material of construction: SS/PTFE

ii. Supercritical CO_2Generator - Material of Construction: SS 316

iii. Extraction Vessels - Number of Extractors: 2
- Volume: 1L (each one)
- 42cm height and 5.5cm inside diameter
- Design pressure 35MPa

- Maximum operating pressure of 30Mpa
- Design temperature 80^0C
- Material of Construction: SS 316
- Design Code: ASME Sec. VIII Div. II

iv. One Pressure Reduction Valve (Material of Construction: SS 316)

v. Low pressure Separators
- No of Separators: 2
-Volume : 1L (each one)
- Design pressure 30MPa
- Maximum operating pressure of 25Mpa
- Material of Construction: SS 316
-Design Code: ASME Sec. VIII Div. II

The other ancillary components of this unit are-

i. CO_2 cylinders (Solvent CO_2 feed vessel) - Purity of CO_2: 99.99%
- Quantity of CO_2: 35kg

ii. A refrigeration unit (Design lowest temperature -15^0C)

iii. Solvent CO_2 Storage Vessel

iv. Piping, Valves & High Pressure Regulator (Material of Construction: SS 316)

v. Heating Jackets surrounding the extraction vessels, supercritical fluid generator, and separators to keep the temperature constant to the set point.

vi. Control Unit to view and change the system settings

vii. Fittings: Swagelok

viii. Isolation valves, Safety Valves for protections.

The description of the SFE solid-fluid system "CSL/SCF/1L2/400" may be summarized as – It is furnished with one high-pressure pump for liquefied CO_2 delivery, one SCO_2 generator unit, two parallel-connected 1L extraction vessels (E-I & E-II), one pressure reduction valve and two series-connected low pressure separation vessels (S-I & S-II) of equal capacity of 1L. The temperatures of supercritical CO_2 generator, extraction vessels, separators, and chiller are adjusted as per experimental design using "Control Unit." The pressure of extraction is maintained using a high pressure regulator valve.

The inlet and outlet of extractors and separators are provided with pressure gauges to ensure correct pressure settings. The variation of temperatures to set point was observed only $\pm 2^0$C. The variation of pressure (equal to ±0.5MPa) in both extraction and separation vessels were under control.

The actual experimental setup of SFE is shown in the Fig.4.1 and labeled parts are listed below. The schematic of the supercritical CO_2 extraction setup was as shown in Fig. 4.2.

Fig.4.1: Semi-continuous SCO_2E Experimental Setup (Model No: CSL/SCF/1L2/400)

1. CO_2 Cylinder
2. Refrigerated CO_2 Storage Vessel
3. High-pressure Pump
4. Supercritical CO_2 Generator
5. Extractor I (E-I)
6. Extractor II (E-II)
7. High Pressure Regulator
8. Separator I (S-I)
9. Separator II (S-II)
10. Control Unit
11. Pump Head Cooling System

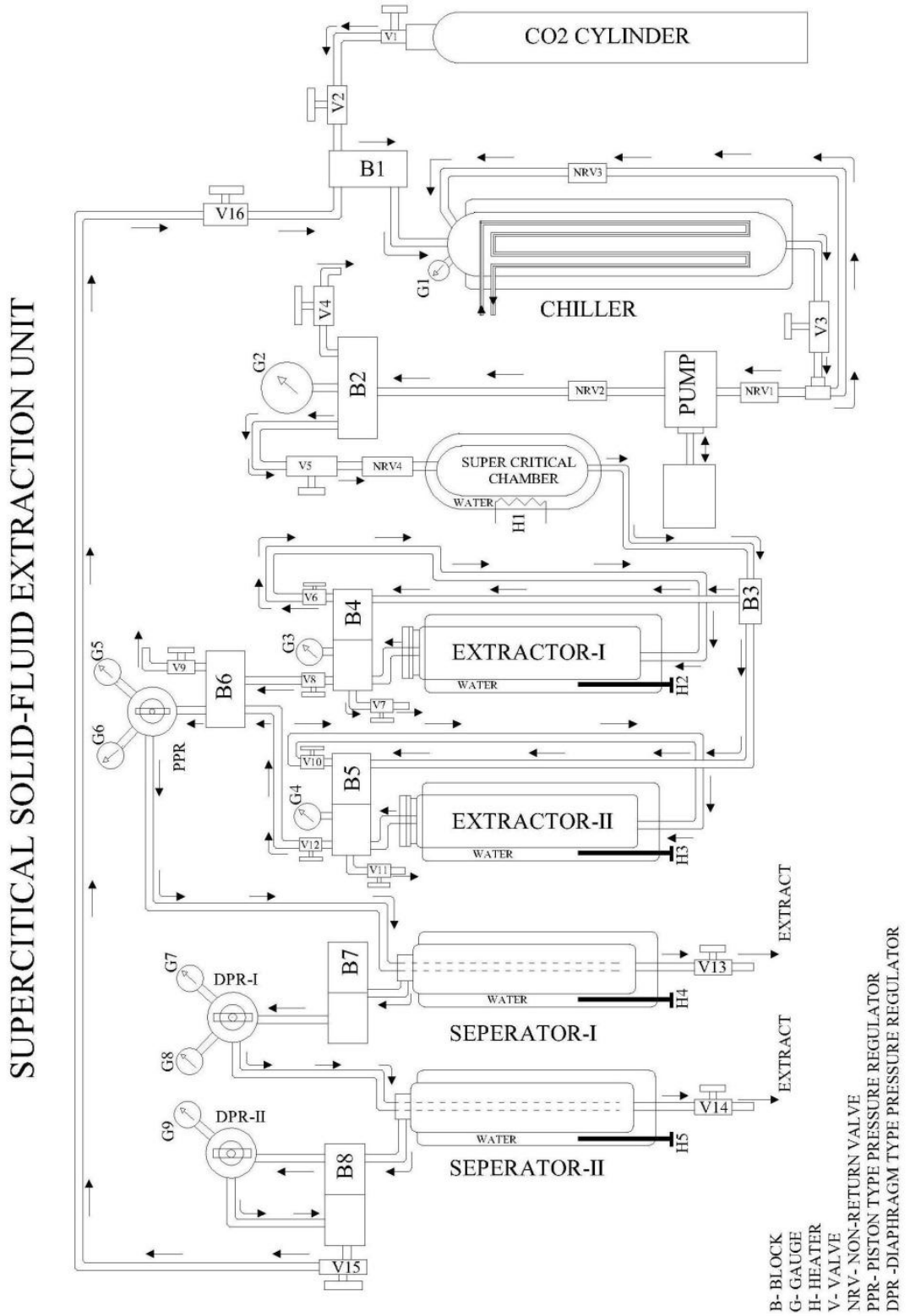


Fig. 4.2: Schematic of Supercritical CO_2 Extraction Setup

4.5.1 Design of External Extraction Feed Vessel for Supercritical Carbon Dioxide Extraction:

Conventionally the solid feed samples before extraction are packed inside the extraction vessel with the help of an externally loaded cylindrical shell having a perforated surface (1 mm perforation) and transferred inside the main extraction vessel inbuilt with the mother unit. The dimensions of this external shell are identical with the extraction vessel of the mother unit (i.e., Volume: 1L, Height: 42cm and Diameter: 5.5cm). The diagram of the conventional cylindrical feed vessel is shown in Fig. 4.3.

Fig.4.3: Conventional Cylindrical Feed Vessel (B1)

In this study along with conventional cylindrical feed vessel, a special modification in bed geometry was introduced by placing a small diameter concentric tube inside the outer larger diameter (5.5cm) cylindrical shell. This concentric tube has perforated surface, same height like the larger shell and one blind end at the upstream side. In this way, conventional cylindrical bed geometry was changed into annulus geometry. Two different diameters concentric tubes (0.75cm and 1.5cm) were chosen to design the annular bed geometry. The dried ground solid sample of plant materials was filled in the annular space of the modified bed and transferred in the main extraction vessel likewise conventional cylindrical shell.

The influence of bed geometry on extraction performance was studied using three different geometry configurations –

i. Conventional cylindrical solid feed bed without any internal annular path (B1)
ii. Annular solid feed bed using 0.75cm diameter inside channel (B2)
iii. Annular solid feed bed using 1.5cm diameter inside channel (B3)

The diagram of concentric perforated channels along with conventional cylindrical feed bed is shown in Fig. 4.4.

Fig. 4.5 shows clearly the created annular geometry by placing the concentric tube inside the conventional cylindrical bed.

Fig. 4.4: Concentric Channels of 0.75cm and 1.5cm Diameters and 5.5cm Diameter Cylindrical Shell

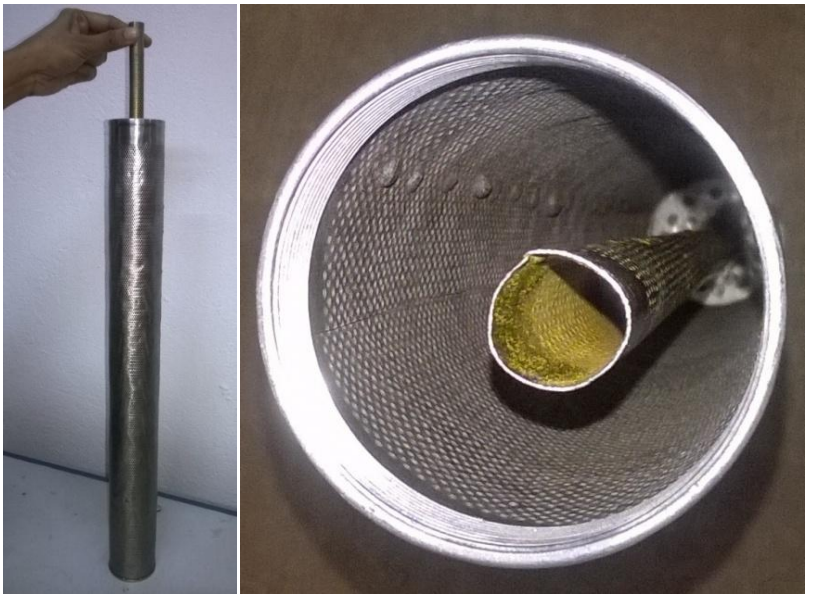
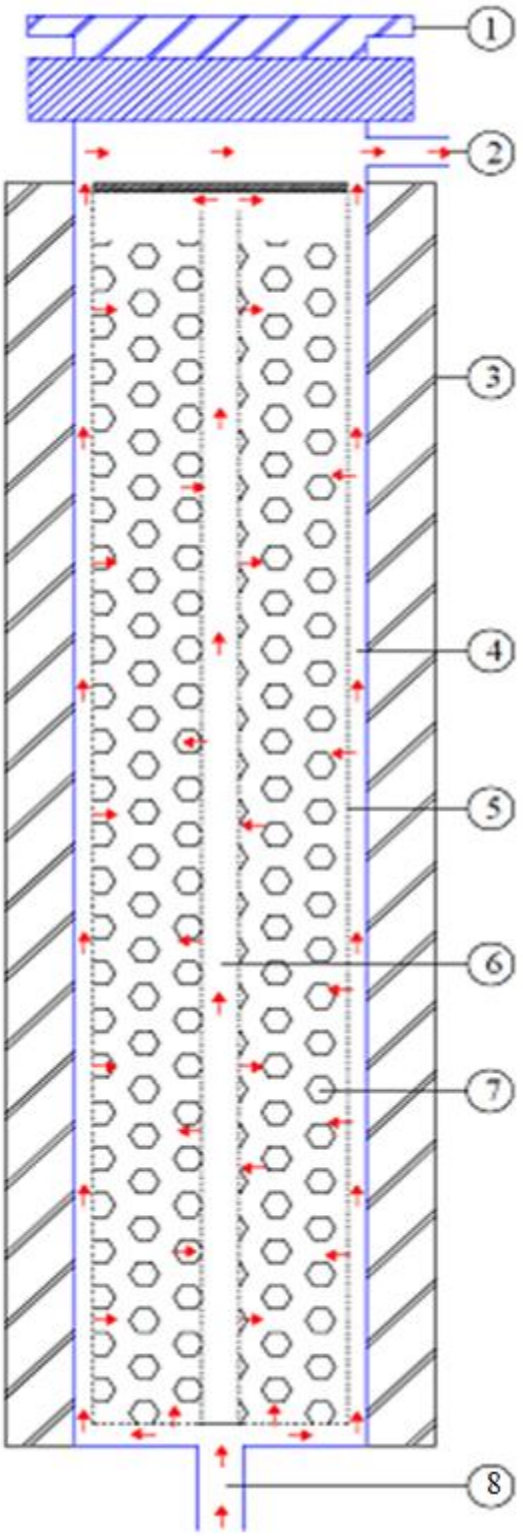


Fig.4.5: Centrally Placed Inner Channel into the Conventional Bed to Create Annular Geometry

The schematic of an annular solid feed bed loaded inside the main extraction vessel is shown in Fig. 4.6.



1. Extractor cap
2. Solute (essential oil) rich supercritical carbon dioxide
3. Thermostatic water bath
4. Extractor vessel
5. Perforated annular feed bed
6. Perforated concentric tube
7. Ground botanical mass
8. Supercritical carbon dioxide

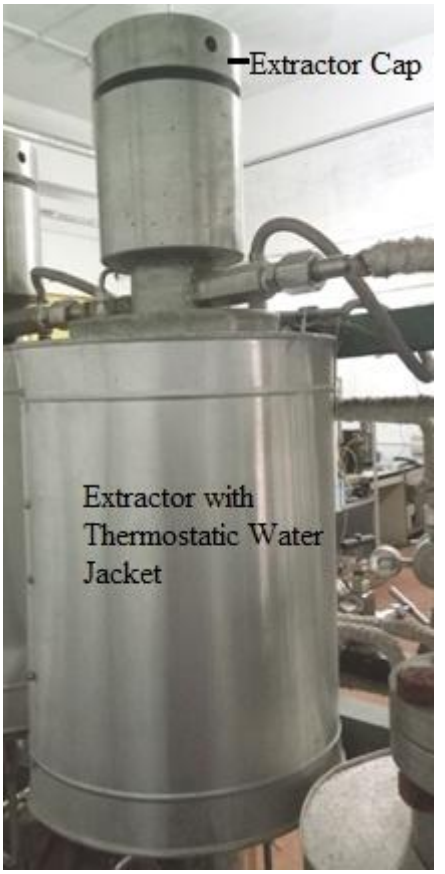


Fig.4.6: Schematic of Annulus Extraction Vessel of SFE Module

In order to study the SCO_2E for smaller size particles of the plant material, cylindrical shell of fine mesh was prepared from the sheet of Mesh No. 200. The diagram of the conventional cylindrical feed bed of varying perforation and concentric channels are shown in Fig. 4.7.

Fig.4.7: Modification of the Cylindrical Feed Shell for Fine Size Particles

4.6 Experimental Procedure of Supercritical Carbon Dioxide Extraction:

The primary steps involved in SCO_2E of plant materials are –

i. Loading the Extraction vessels with plant materials,
ii. Solvent CO_2 Conditioning,
iii. SCO_2 Generation,
iv. Extraction
v. Expansion,
vi. Separation
vii. Solvent Recovery.

The detail experimental procedure is described here following the schematic of supercritical carbon dioxide extraction set up Fig. 4.2.

For extraction experiment initially, one particular type of feed vessel among the above mentioned three types (B1 or B2 or B3) was chosen and packed externally with dried crushed plant materials while both ends of the bed were blocked with polypropylene wool to prevent any solid loss and protect the extractor lines from blockage. This external vessel filled with solid material was then placed properly inside the main extraction vessel (anyone of E-I or E-II) and top of the extraction vessel was closed carefully with the extractor cover using hand pressure. A small leakage in the top cover of the extraction vessel has resulted in the failure of the experiment.

Before starting the experiment, the temperatures of the SCO_2 generator, extractor, and separators were set using a control board based on experimental design and allowed to rise to the set point. The CO_2 cylinder pressure was checked to confirm that it was above 5MPa to get required supply. Opening the valves V1 and V2, liquid CO_2 was allowed to enter in the solvent storage vessel whose pressure was monitored to maintain in the range from 6.0MPa to 7.0MPa to get sufficient flow of solvent. The chiller was run to reduce the solvent temperature in the storage vessel below 5^0C.

Once these above mentioned conditions were attained, the main extraction process was carried out following all the steps discussed below-

i. The liquefied solvent CO_2 from the bottom of the refrigerated solvent vessel was flowed through the valves V3 and NRV1 (Non-return valve, NRV) and pressurized to the desired extraction pressure (above the critical pressure of 7.39MPa) with the help of high-pressure plunger pump. The heat generated in the pump head was lowered with the help of a cold water circulation system through which chilled water below 5^0C was continuously flowing (as shown in Fig. 4.8).

Fig. 4.8: High Pressure Pump Head Cooling Water Circulation System

ii. Pressurized liquid CO_2 was allowed to enter the SCO_2 generator unit, which is built with a thermostatic water bath. Here pressurized CO_2 heated up to attain the desired supercritical temperature (which is above critical temperature 31.1^0C) of extraction. In this way, solvent CO_2 was conditioned to acquire favourable supercritical condition.

iii. Now supercritical CO_2 entered into the solid loaded extraction vessel (either E-1 or E-2) where the desired extraction pressure was allowed to develop closing the outlet valve of the extractor (V8 or V12). The system was kept in this condition to provide a static extraction period (ts) of 20 minutes before starting the dynamic phase of extraction to ensure better contact between fluid-solid phases in all the experiments performed. Throughout the dynamic

period of extraction, CO_2 was circulated through the whole system and designed operating conditions of extraction were maintained. The extractor pressure was controlled by manually operated high pressure regulator (PPR in Fig 4.2). As the nature of oil varies for different plant materials, the extraction was continued for different times for clove and turmeric sample to ensure the required degree of recovery.

In the extractor, pressurized CO_2 dissolves the oil molecules exposed to the surface quickly due to high solvation power like liquid and penetrates through the fine particles of plant materials like a gas to extract their essence and diffuses back.

iv. The extract rich solvent after leaving the extractor vessel was allowed to expand through two successive extract recovery vessels termed as Separator-I (S-I) and separator-II (S-II). In these two steps the temperature and pressure of supercritical solvent were reduced to that extent (below 31.1^0C, and 7.39MPa pressure) so that SCO_2 converted back into gaseous form. Thereby, the solvation power of the solvent CO_2 for solute oil molecules drops significantly and oil is separated from the carrier CO_2 and settled down in the bottom of S-I and S-II.

v. The extracted oil was drained out from the bottom of the separator vessels. The collection valve of the separator should be opened slowly with care to avoid any sudden spillage of oil due to release from high pressure. In all the experiments, the oil samples were collected at intervals using different sampling bottles, weighed separately using precision balance (Citizen CTG 602) and recorded to prepare OEC.

vi. After the second stage of separation, oil-free solvent CO_2 as gaseous form is recovered from the top of the Separator-II with the help of a recycle system by opening valves, V-15 and V-16 while closing main supply valve of CO_2 i.e., V-2 and returned back to refrigeration section for re-circulation.

vii. After completion of the extraction process, the inlet and outlet valves of extraction vessel were closed tightly and it was vented completely to depressurize. Then opening the extractor cap, the external feed bed was unloaded to remove the oil extracted solid materials. Thus solvent CO_2 present in the other sections remains unaltered.

viii. Finally, the total oil extract was centrifuged and separated from other co-extracts (present in negligible quantity) and stored under refrigeration until the analyses were carried out.

The oil recovery is expressed as the ratio of the percentage of oil yielded to the mass of solid feed used for extraction by using the formula as given below:

$$Percentage\,oil\ yield,\ \%OY = \frac{Weight\ of\ oil\ extracted\ (gm)}{Weight\ of\ dried\ sample\ used\ to\ extract\ oil\ (gm)} \times 100 \quad \text{-------(4.1)}$$

It has been understood from the literature search that the crucial parameters along with extractor bed geometry, which influences the supercritical CO_2 extraction processes are temperature, pressure, particle size of plant materials, etc. Effect of these parameters for extracting essential oils from clove buds and turmeric rhizomes using external feed bed of varying geometry were studied in successive chapters.

Fig. 4.9 shows the process flow chart of supercritical carbon dioxide extraction of plant materials to produce essential oils.

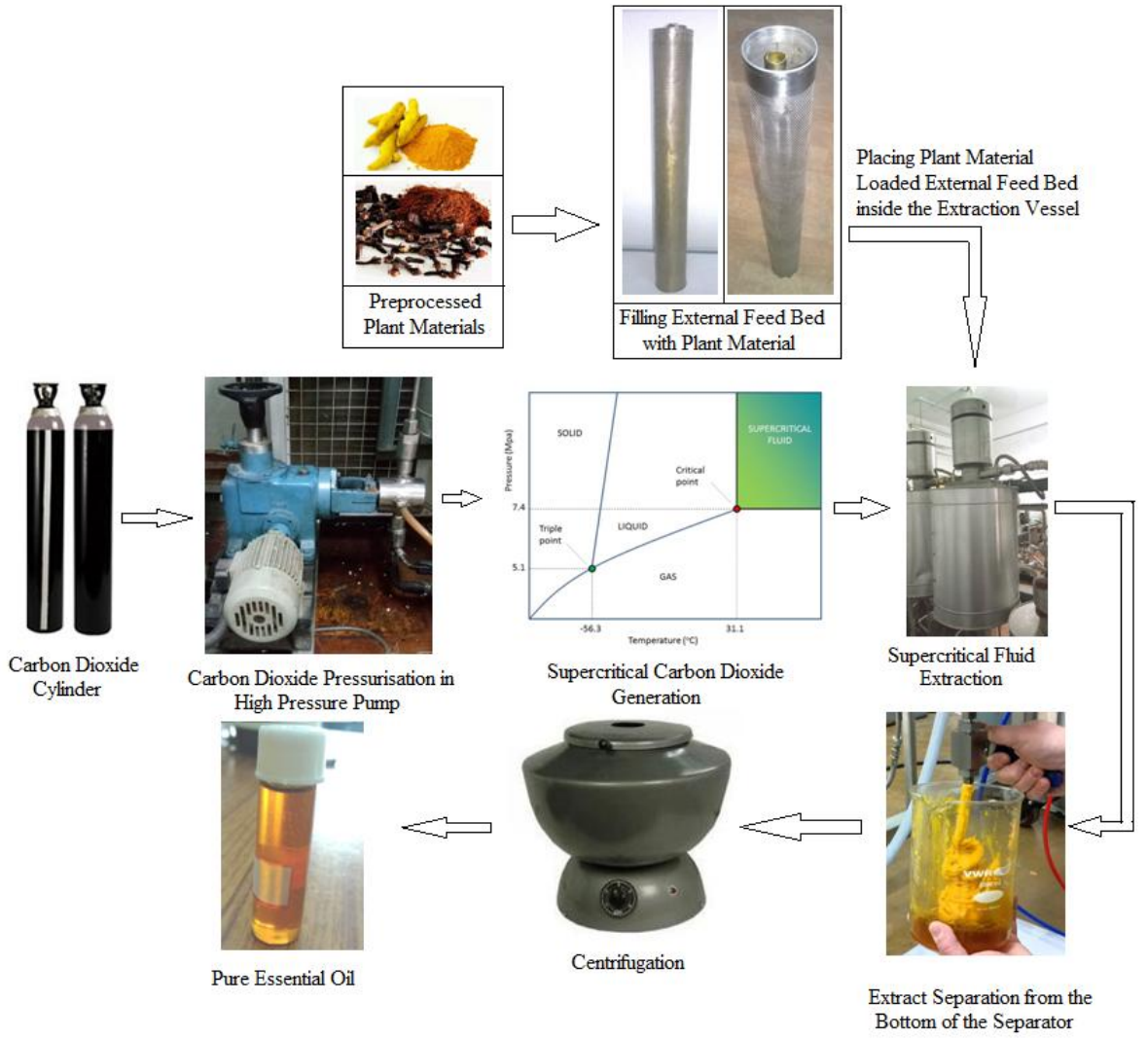


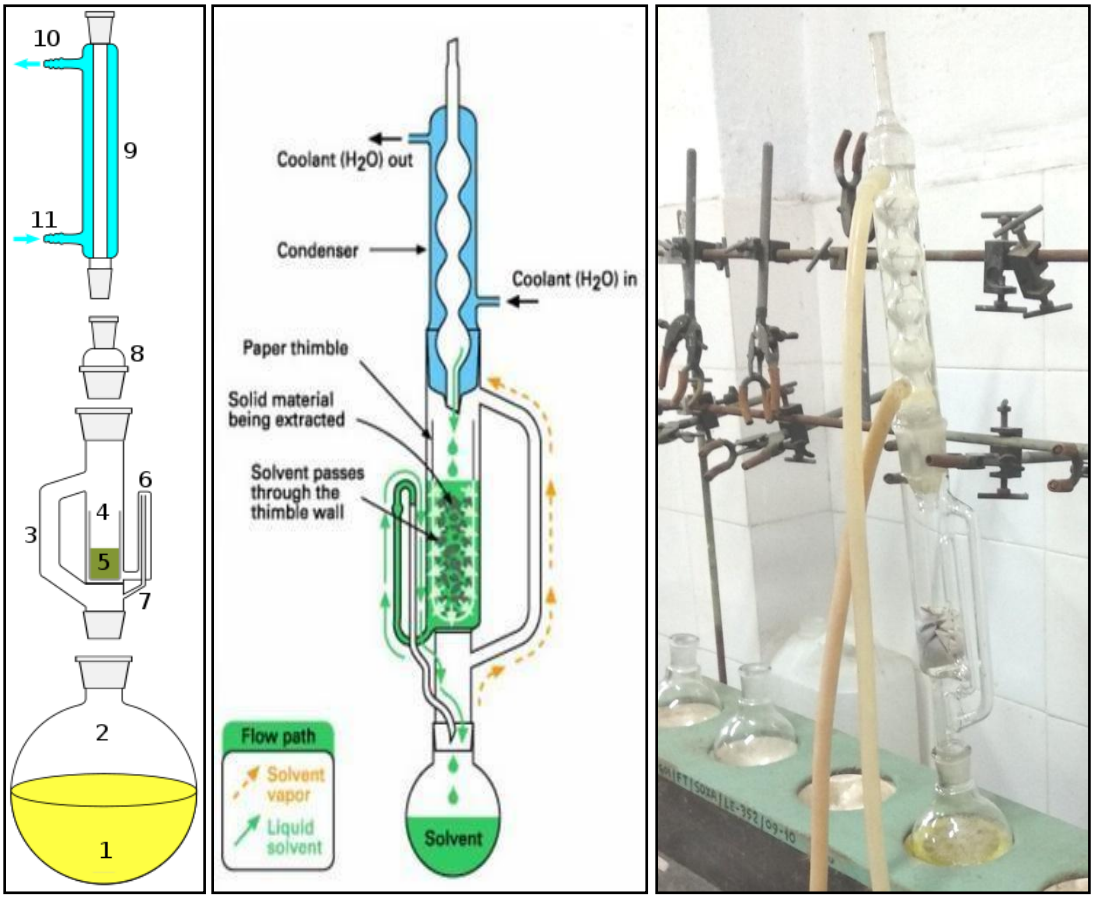
Fig. 4.9: Process Flow Chart of Supercritical Carbon Dioxide Extraction

4.7 Soxhlet Apparatus for Solvent Extraction:

As per literature search, Soxhlet extraction has been considered as a standard technique and the main reference to determine the performance level of other techniques used for solid-liquid extraction processes in terms of quantity and quality of extract. In the present study, the traditional Soxhlet extraction method was applied to obtain the total extractable soluble part from the aromatic plant samples of clove buds and turmeric rhizomes. The yield obtained by this method was termed as "Global Yield."

The laboratory apparatus for Soxhlet extraction was supplied by Zenith Glass wares and Instruments Corporation, Kolkata (developed following the inventor Franz von Soxhlet's set up). It was made of Borosilicate Glass. Its design temperature was 250^0C.The various sections of a

standard Soxhlet Apparatus are listed below based on the leveling of the set-up. Fig. 4.10 represents schematic diagram as well as experimental set up of a Soxhlet Apparatus used for this study.



(A) (B) (C)

(A):Schematic of a Soxhlet Apparatus showing Different Parts (Source:https://en.wikipedia.org/wiki/Soxhlet_extractor)

(B): Schematic of a Soxhlet Apparatus showing Flow Path of Solvent Vapor and Solvent Liquid (Source: Asu Inan, 2013)

(C): Experimental Set-up of Soxhlet Apparatus for Solvent Extraction

1: Hydrocarbon Solvent
2: Round Bottom Reflux Flask
3: Distillation path
4: Thimble
5: Solid feed sample
6: Siphon top
7: Siphon exit
8: Expansion adapter
9: Condenser
10: Cooling water out
11: Cooling water in

Fig. 4.10: Schematic of Soxhlet Apparatus

4.8 Experimental Procedure of Soxhlet Extraction :

Soxhlet extraction involves contact between liquid solvent and solid material for the separation of soluble components from the solid sample by dissolving them into a refluxing liquid pool. At first dried ground plant matrix was wrapped in a filter paper and placed into the main chamber of Soxhlet extractor. The required volume of selected solvent was filled in the round bottom reflux flask and the flask was placed over a heating mantle. One end of the Soxhlet extractor was connected with the flask and the reflux condenser was placed atop the other end of the extractor.

Once the setup was ready to conduct extraction, the solvent was heated up with the help of heating mantle. The solvent vaporized and traveled up the distillation side arm and contacted with condenser section. The condenser ensures liquefaction of the solvent vapour and prevents any solvent loss. The condensed liquid dripped down into the main extractor chamber and contacted with the solid sample where solvation of the desired compounds in the warm solvent started. With time the liquid pool started to develop slowly into the extractor chamber and when reached the overflow level, the chamber is completely emptied by the siphon side arm. The solvent loaded with its extracted analytes is returned to the distillation flask and mixed with the bulk liquid. Filter paper thimble prevented any solid loss in this process. This operation of solvent recycling is repeated until complete extraction was achieved.

After many cycles when the desired components were concentrated in the solvent, the distillation flask was removed from the extractor system and the solvent was allowed to evaporate in a rotary vacuum evaporator to obtain concentrated yield. Then residual oil was cooled at room temperature and weighed using precision balance (Citizen CTG 602).The insoluble portion of plant material was removed from the extraction chamber and discarded.

The oil recovery was expressed as percentage oil yield with respect to the mass of solid feed used for extraction by using Eq. (4.1).

$$Percentage\ oil\ yield,\ \%OY = \frac{Weight\ of\ oil\ extracted\ (gm)}{Weight\ of\ dried\ sample\ \ used\ to\ \ extract\ oil\ (gm)} \times 100 \text{ -------(4.1)}$$

4.9 Analytical Methods

4.9.1 Thermogravimetric Method for the Determination of Moisture

The moisture content of ground plant materials was determined using SARTORIUS MA45 moisture analyzer, which works based on the thermogravimetric method (Fig. 4.11). The material was distributed uniformly over a Sartorius disposable sample pan (Order No. 6965542, inner diameter = 92 mm) in a thin layer of 2 to 5mm height in order to avoid any uneven heating or drying or burning of the sample or elongated period of drying. As some bioactive components are heat sensitive, the sample was covered with a glass fiber filter (order no. 6906940). The sample pan was placed over a pan draft shield with the help of a support. Once the sample was prepared for drying, the sample chamber was closed, and the heating and drying of the sample were started in a very gentle and uniform way. It can produce quick and authentic results with high

repeatability thus save time and workloads. After performing the analysis, the pan draft shield was removed from the sample chamber to clean the weighing system from debris.

(A) : Laboratory Moisture Analyzer Set-up

(B) : Disposable Aluminum Sample Pans

(C) : Solid Sample over Sample Pan

Fig. 4.11: Moisture Analyzer Analytical Instrument

4.9.2 GC-MS Method for Chemical Analysis of Essential Oils

Chemical analysis of essential oils was performed to determine the qualitative and quantitative natures of the oils in terms of the bioactive components present in it. The method of analysis was based on chromatographic with a flame ionization detector and spectroscopic criteria. Sample of essential oil containing multiple constituents was vaporized with the application of heat at the GC, and vaporized molecules were carried through a column for separation with an inert carrier gas. As the separated constituents come out from the column opening, they were allowed to flow into the MS. MS ionized the component molecules, separated them according to their mass, and then identify and quantify. A GC-MS can measure any molecules or minute constituents from nanogram to femtogram levels.

In the present study, an advanced standard gas chromatograph-mass spectrometer (GC-MS) was used for essential oil analysis. It consists of a gas chromatograph (Model GC-2010 Plus) for separation of the components and a mass spectrometer (Model GCMS-QP2010 SE) supplied by SHIMADZU, Kyoto, Japan for identification and quantification of the components (Fig. 4.12). This GC-MS is equipped with DB - 1 MS UI capillary column (Specifications: length 60m, inside diameter 0.25mm, internal film width 0.25µm) supplied by Agilent to analyze the sample components. The sample of essential oil was diluted using acetone in 1:4 ratios (1% oil and 4% acetone) for chromatographic injection and 1 µL volume of sample was injected with the help of auto-sampler in the split mode (1:50). The injection temperature was 230^0C. The carrier gas used in GC was helium (He). It was allowed to flow maintaining the conditions of (i) total flow 53.3 mL/min, (ii) column flow 0.50 mL/min, (iii) purge flow 3.0 mL/min and (iv) pressure 63.3kPa. GC oven temperature was gradually increased starting from 50^0C, maintained for 3min, and then increased successively at the rate of 1^0C min^{-1} for 10 minutes, 2^0C min^{-1} for 40 minutes and 3^0C min^{-1} for 30 minutes until 230^0C attained, maintaining isothermal condition for last 7 minutes. The total run time was 90 min.

Mass spectroscopic detectors (MSD) are the most powerful gas chromatography detectors. It was directly connected with the capillary column to receive the mobile phase leaving from the capillary column. EI mode of ionization was used in this MS. The settings used in the MSD were – (i) Ion source temperature 220^0C, (ii) interface temperature 300^0C.

The signal produced by the MSD is the chromatogram, which is a plot of signal magnitude vs. time. The obtained mass spectra can be matched using GCMS solution software (version 4) developed with MS library - NIST, Wiley, and SHIM to identify the peaks. Based on the area of peaks as obtained in the chromatogram of the clove oil sample, the quantity of the components was calculated individually. All the testing of samples was done in the quality control laboratory of Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

Fig.4.12: Gas Chromatograph - Mass Spectrometer Analytical Instrument

4.9.3 Spectrophotometric Method for the Determination of Antioxidant Activities

Different Antioxidant Power assays or free radical scavenging activity assays of plant essential oil extracted using SCO_2 as a solvent were determined following the standard Spectrophotometric methods using an UV- Spectrophotometer (Shimadzu UV-vis 1800 Spectrophotometer). Fig. 4.13 shows the UV-Spectrophotometer Analytical Instrument used in the present work.

Fig.4.13: UV-Spectrophotometer Analytical Instrument

Chapter 5

EXPERIMENTAL DESIGN OF SCO_2 EXTRACTION OF CLOVE & TUMERIC

5. Introduction

The Literature search reveals that the various parameters that may influence the performance of essential oil extraction using SCO_2 are pressure, temperature, particle size, solvent flow rate, the use of co-solvent, moisture level, extraction time, plant material drying conditions, the maturity of biomass, and others [Rai, 2015]. Another critical factor that has a significant influence on the extraction efficiency along with these operating parameters is the extractor design configuration. The variation in geometric alteration in extractor design interferes in its performance by affecting the distribution pattern of solid feed, the tortuous path for solvent flow, mass and heat transfer rates and cost of extraction vessel [Zabot, 2012]. All these variables influence the overall extraction curves (OEC) and corresponding extraction kinetics, yield, quality of extract, and cost involved with the extraction process.

One of the main features that should be considered to maximize the desired product quality and the process yield, minimize the undesired one and the cost of SCO_2E process is the extraction optimization. The process of optimization makes it possible to find out the optimum conditions of the processing variables that give the optimum value of the desired product within the boundary of the variables. The use of these optimum conditions of the different parameters influencing the SCO_2E could significantly enhance the extraction yield of essential oil as well as recovery of targeted compounds. The independent or interactive effects of the variables under consideration for optimization are also possible to determine during the optimization process.

Extraction optimization operations follow some particular methodologies that involve the formulation of a mathematical model that represents desired outcome or response (such as, yield or targeted component) in terms of different variables (such as, process parameters such as temperature, pressure, solvent flow rate etc.) based on time-efficient experimental design and their execution. These are known as experimental optimization. Since SFE experiments are generally expensive and time consuming, planning of experiments following suitable optimization methodology is of primary importance as it directs to conduct the least number of experiments to frame the objectives satisfactorily and thus save time, reduce consumption of raw materials, chemicals, energy, etc. [Montgomery, 2001].

Experimental optimizations use mathematical and statistical techniques to optimize the extraction process. The response surface method (RSM) is a powerful tool of statistical design, analysis and optimization that is most commonly used in the SCO_2E researches since it considers various possible interactions of the variables during optimization [Haaland, 1989] and find out the best combination of the factors to achieve the highest response (yield or quality of yield). Application of RSM in industrial research where it is required to handle a large number of variables affecting the process is extensive [Lazic, 2004; Nwabueze, 2010].

For the design of the experiments, various techniques were developed under RSM. They are-

i. Three-level factorial design (FD)
ii. Central composite design (CCD)
iii. Box-Behnken design (BBD)

Among these CCD provides less number of tests for the given variables and central points and also recommended or preferred for developing effective model [Granato, 2014; Priyanka, 2014].

This chapter represents parametric studies of SCO_2E of clove buds and turmeric rhizomes using various external bed geometry configurations as discussed in the previous chapter for process optimization, determination of process kinetics and evaluation of suitable extraction model.

5.1 Supercritical Carbon Dioxide Extraction of Clove Buds

5.1.1 Preprocessing of Raw Materials

The quality flower buds of clove collecting from the local market of Haldia (West Bengal, India) checked thoroughly for removal of foreign substances (if any) and dried at 30°C in a laboratory scale air circulated drying unit (Supplied by M/S Instrumentation India, Kolkata) for 6 h. This moisture treatment was performed to reduce the moisture level of the raw feed material below 12% to avoid the negative impact of moisture on the mass transfer rate and solubility of volatile matter in the solvent CO_2 [Goodrum, 1987; Ivanovic, 2011].

The dried clove buds were then ground into smaller size particles in a mixture grinder (Philips Mixer Grinder HL7720). The ground material was then passed through sieves from Tyler standard screen series to classify the ground clove sample into three different particle sizes. For this classification, sieves of three ranges of mesh sizes were used: 10-14, 14-20, 20-42.The particle size (D_P) was determined following the mass mean diameter calculation.

Fig. 5.1: Preprocessing Steps of Clove Buds before SCO_2E

The ground mass was packaged in air-tight polyethylene bags and stored under refrigeration of 4^0C for subsequent experimental studies. The light and heat sensitive components loss may be enhanced on exposure to light and heat because of smaller particles with larger surface area after grinding. Hence, the sample was stored in dark, cold places until further experiments start. The preprocessing steps of clove buds are shown in Fig. 5.1.

5.1.2 Moisture Content

The moisture contents of raw ground clove sample, as well as dried ground clove sample, were determined as described in section 4.9.1 using "SARTORIUS MA45C" moisture analyzer (Fig. 4.11). The moisture analysis results are prepared by averaging the results obtained from triplicate measurements.

5.1.3 Determination of Total Amount of Extractable Matter (Global Yield)

The total amount of extractable matter or global yield present in clove sample was determined using conventional Soxhlet extraction method as described in section 4.7. Approximately 30±0.1 gm of preprocessed dried ground clove sample (from the same feedstock that was intended to use in SCO_2E studies)of 0.64mm particle size was wrapped in a Whatman filter paper thimble and placed inside the main extractor chamber of the Soxhlet apparatus. Then the bottom end of the Soxhlet extractor was connected with round bottom reflux flask of 500 mL capacity and the upper end was attached with the condenser system. Starting water circulation through condenser system and supplying heat at the bottom of the distillation flask, extraction experiment was carried out using 300 mL n-hexane for 6hrs to ensure complete separation of soluble matters. The clove oil extract was concentrated by evaporating solvent at 50^0C with the help of a rotary vacuum evaporator. Then residual oil was cooled at room temperature and weighed using precision balance (Citizen CTG 602).The reported value of global yield is the mean of three replications.

The oil recovery was expressed as percentage oil yield with respect to the mass of solid feed used for extraction by using Eq. (4.1).

5.1.4 Experimental Design to Optimize SCO_2E process

The aims of any essential oil extraction process are increasing the yield of oil and concentration of important bioactive components in the extract in a cost-effective method. For achieving this, process optimization considering important factors that affect yield and desired compounds is required selecting a suitable methodology. In this study, a mathematical relationship among the variables was proposed with the help of RSM of statistical analysis to optimize three critical factors namely bed geometry (X1), temperature (X2) and pressure (X3) towards maximization of clove oil yield by the method of SCO_2E. The modified extractor bed geometry was given particular importance in this investigation to evaluate its effect on clove oil extraction and optimum value under design consideration. The outcome of the RSM analysis that was

represented as a function of these three variables is the percentage oil yield expressed by simplification of Eq. (4.1) as –

$$Percentage\,oil\ yield, \%OY = \frac{Weight\ of\ oil\ extracted\ (gm)}{100\ gm\ of\ dried\ sample\ used\ to\ extract\ oil} \quad \text{---------- (5.1)}$$

The bed geometry (X1) of extraction vessel was expressed in this analysis in a simplified dimensionless form as axial to radial surface enhancement factor (ARSEF). ARSEF is defined as the ratio of axial surface or cross-sectional area [i.e., $\pi(r_o^2-r_i^2)$] to radial surface area [i.e., $2\pi L(r_o+r_i)$]. The final form of ARSEF as obtained from this ratio is given below-

$$X1 = ARSEF = \frac{(r_o - r_i)}{2L} \quad \text{----------(5.2)}$$

where r_o is the radius of the outer larger cylinder, r_i is the radius of the inner cylinder, and L is the length of the external feed bed.

The r_o and r_i values and corresponding calculated values of ARSEF for three different bed B1, B2, and B3, as discussed in Section 4.5.1 are given in Table-5.1.

Table-5.1: Calculations of ARSEF for Three External Feed Beds

Bed Type	r_o (cm)	r_i (cm)	L (cm)	$X1 = ARSEF = \frac{(r_o - r_i)}{2L}$
B1	2.750	0.000	42	0.0327
B2	2.750	0.375	42	0.0283
B3	2.750	0.750	42	0.0238

In the present study, face-centered central composite design (FC-CCD) strategy was chosen for the experimental design of these three factors to obtain the %OYs as responses from different tests conducted for various combinations of these factors. Three levels of these three independent variables (X1, X2, and X3) were coded as (−1), (0) and (+1) following general rules and are represented here in Table-5.2.

Table-5.2: Three levels of selected variables chosen for FC-CCD under RSM

$ARSEF,\ X1 = \frac{(r_o - r_i)}{2L}$	Temperature, X2 (^{0}C)	Pressure, X3 (MPa)
B3 → 0.0238 (-1)	T1 → 35 (-1)	P1 → 14.7 (-1)
B2 → 0.0283 (0)	T2 → 40 (0)	P2 → 19.6 (0)
B1 → 0.0327 (+1)	T3 → 45 (+1)	P3 → 24.5 (+1)

FC-CCD generated twenty different combinations of these three independent variables i.e., the volume of experiments that were planned based on these twenty combinations to find out responses was twenty.

From Table-5.2 it is clear that the temperature and pressure levels selected were 35^0C (-1), 40^0C (0), and 45^0C (+1) and 14.7 MPa (-1), 19.6 MPa (0), and 24.5 MPa (+1), respectively. The minimum extraction temperature of 35^0C was chosen to raise the temperature of solvent CO_2 above its critical value (31.1^0C), and the maximum temperature of 45^0C was set following the reported value of optimum temperature of 40^0C for clove oil [Prado, 2011]. Since extraction of clove oil from its matrices is easier at moderate temperature, higher temperature was not selected to avoid the extraction of unwanted compounds and possible thermal degradation of the extract. Regarding the extraction pressure, the minimum value of 14.7 MPa was chosen considering the positive influence of pressure on density which is mentioned proportionally related with the solubility of the solvent CO_2 at all levels [Mukhopadhyay, 1998;Frohlicha, 2019]. The maximum extraction pressure was selected as 24.5 MPa according to the study of Mukhopadhyay and Rajeev, 1998, which revealed that increasing pressure results into a significant increase in the rate of extraction and thus reduces the extraction time for the desired degree of separation. Three values of the dimensionless parameter X1 used in this study were 0.0238(-1) for annular bed (B3), 0.0283 for annular bed (B2) and 0.0327 for conventional cylindrical feed bed (B1) as listed in Table-5.1.

After fixing extraction variables and responses for optimization to meet the objectives of the present study, statistical software package Design Expert-11 was applied to generate the FC-CCD based experimental settings of three variables to be used while performing the experiments. Once all the experiments using clove sample were carried out following the FC-CCD design, the responses (% OY) were entered in the appropriate column of the design expert's design layout. Then the responses were analyzed numerically to fit the experimental response data with the input factors in terms of different mathematical models (such as linear model, factorial models, quadratic model, etc.) by this software. The statistical significance of these models was tested, and model with insignificant lack of fit and lower standard deviation value was suggested by the inbuilt program of Design Expert in the model fit summary report section. Finally, in-depth statistical studies of the suggested and selected model were performed by means of the analysis of variance (ANOVA) which confirms the adequacy of the model and final form of the equation in terms of the input variables for the predictive model was also obtained at the end of ANOVA analysis. This equation is very much helpful to predict the responses for each input factor within its test range [Iwe, 2004]. For a complex system, the relationship between any two input variables and one response variable may form a curvature in their plane. The quadratic or higher polynomial model should be proposed as a suitable fit [Nwabueze, 2010]. The influence of each independent factor and their interactions were examined and estimated statistically during ANOVA, which are explained by graphical means in the results section.

The data set of three independent variables with their coded levels as generated by FC-CCD is tabulated in Table-5.3.

Table-5.3: Experimental design for SCO_2E of Clove Buds as per FC-CCD

Run No.	Input Factors		
	$ARSEF, X1 = \frac{(r_o - r_i)}{2L}$	Temperature, X2 (^{0}C)	Pressure,X3 (MPa)
1	0.0283 (0)	45(+1)	19.6 (0)
2	0.0238 (-1)	35(-1)	24.5 (+1)
3	0.0283 (0)	40(0)	19.6 (0)
4	0.0283 (0)	40(0)	24.5 (+1)
5	0.0327 (+1)	35(-1)	24.5 (+1)
6	0.0238 (-1)	45(+1)	24.5 (+1)
7	0.0327 (+1)	40(0)	19.6 (0)
8	0.0283 (0)	40(0)	19.6 (0)
9	0.0283 (0)	40(0)	19.6 (0)
10	0.0238 (-1)	40(0)	19.6 (0)
11	0.0327 (+1)	45(+1)	14.7 (-1)
12	0.0283 (0)	40(0)	19.6 (0)
13	0.0283 (0)	40(0)	14.7 (-1)
14	0.0238 (-1)	35(-1)	14.7 (-1)
15	0.0238 (-1)	45(+1)	14.7 (-1)
16	0.0283 (0)	35(-1)	19.6 (0)
17	0.0327 (+1)	45(+1)	24.5 (+1)
18	0.0327 (+1)	35(-1)	14.7 (-1)
19	0.0283 (0)	40(0)	19.6 (0)
20	0.0283 (0)	40(0)	19.6 (0)

During SCO_2E experiments of clove buds, all other important parameters such as mass of feed loaded (F), particle size (D_P), solvent flow rate (Q_{CO2}), the initial static period of extraction (t_S), extraction time (t_E), separators temperature and pressure were kept constant. These data are provided in Table-5.4. The extraction procedure using SCO_2 as solvent was followed as per section 4.5. The ground clove buds used in different runs were taken from the same sample preprocessed and stored previously.

Table-5.4: Experimental data of SCO_2E of Clove Buds for RSM Study

F (gm)	D_P (cm)	Q_{CO2} (gm/min)	T in S-I(C^0)	P in S-I (MPa)	T in S-II(C^0)	P in S-II(MPa)	t_s (min)	t_E (min)
600	0.64	18.5	33	≈ 6	28	≈ 5	20	210

Here F - Mass of feed, D_P – Particle size, Q_{CO2} - Solvent flow, T- Temperature, P- Pressure, S-I - Separator I, S-II – Separator II, t_s - Static period of extraction, t_E -Extraction time

5.1.5 Kinetics of Clove Oil Extraction in Different Bed Geometry with SCO_2

Modification of geometric configuration of external feed bed leads to an alteration in the flow dynamics of both solvent and solute molecules within the main extraction vessel. Thus it is an important factor in the design and scale-up of a SFE process. Success of scale up procedure depends on the quality data of kinetic study evaluated from OECs at lab scale or pilot plant scale SFE experimental set up. In this study three different external feed beds B1, B2 and B3 (as discussed in Section 4.5.1) with varying geometries of cylindrical and annulus were used for kinetic studies. Since the annulus bed geometry is an advanced concept in SCO_2E, the evaluation and comparisons of OECs for both conventional cylindrical type and present annulus type geometries are very much relevant for future development.

To develop OECs of SCO_2E of clove buds for kinetic study three different beds (B1, B2, and B3) and three different extraction temperatures (T1=35^{0}C, T2=40^{0}C, and T3=45^{0}C) were chosen to perform the experiments using the same SFE experimental setup. Other parameters such as the mass of feed loaded (F), Particle size (D_P), extraction pressure (P in E), solvent flow (Q_{CO2}), the initial static period of extraction (t_S), extraction time (t_E) and separators pressure and temperatures were kept constant for all runs. These experimental data are furnished in Table-5.5.

Table-5.5: Experimental data of SCO_2E of Clove Buds for Kinetics Studies.

F (gm)	D_P(cm)	Q_{CO2}(gm/min)	T in E (C^0)	P in E (MPa)
600	0.64	18.5	35/40/45	19.6±0.5

T in S-I(C^0)	P in S-I(MPa)	T in S-II(C^0)	P in S-II(MPa)	t_s (min)	t_E (min)
33	≈ 6	28	≈ 5	20	210

Here F - Mass of feed, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, E – Extractor, S-I - Separator I, S-II – Separator II, t_s - Static period of extraction, t_E – Extraction time

A total of 15 clove oil samples were collected and weighted (separately using precision balance, Citizen CTG 602)from each run over the period of extraction initially at an interval of 10 minutes up to 90 minutes followed by 20 minutes interval up to 210 minutes. All the experiments were replicated twice. The yield was expressed as %OY following Eq. (5.1). The experimental OEC data as obtained from various runs were fitted with two straight lines using graphical method and mentioned as constant extraction rate (CER) and falling extraction rate (FER) periods [Prado, 2011; Zabot, G.L., 2014]. Kinetic parameters like (i) constant extraction rate period (t_{CER}), (ii) the rate of extraction during CER (R_{CER}), and (iii)% yield achieved during CER(Y_{CER}), and (iv) % yield recovered w.r.t. the global yield during CER (%Y_{CER}) were evaluated from the OECs. These data along with percentage of total yield (%OY)i.e. the % actual yield obtained over the extraction period of 210 minutes, are reported to compare the performance of various feed bed geometry.

5.1.6 Effect of Particle Size on SCO_2 Extraction of Clove Oil Using Annulus Bed Geometry:

Particle size is an important factor that affects the SFE performance significantly. With changing particle size, the surface area available for mass and heat transfer changes as well as the passage of flow for both solvent and solute also changes. In this study, three different particle size (D_{P1}=0.64mm, D_{P2}=1.0mm, and D_{P3}=1.5mm) and annulus extractor bed geometry (B3) were used to determine the effect of particle size on SCO_2extraction of clove buds. The extraction temperature and pressure were kept constant for all the runs at 45^0C and 19.6±0.5MPa, respectively. Other parameters of extraction experiments were the same as SCO_2E experiments of kinetic studies as given in Table- 5.5.In each run, a total of 15 samples of clove oil were collected over the period of extraction in different time intervals, as stated in section 5.1.4. The yield was expressed as %OY following Eq. (5.1).

5.1.7 Chemical analysis of Clove Oil Extract

The compositions of aromatic bioactive ingredients present in the clove oil samples were identified with the help of an advanced standard gas chromatograph-mass spectrometer, GCMS-QP2010 SE (SHIMADZU, Kyoto, Japan) in the quality control laboratory of Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India. The detail information of GCMS operational procedure, sample preparation manual and inbuilt GCMS solution software with MS library was discussed in section 4.9.2.

5.2 Supercritical Carbon Dioxide Extraction of Dried Turmeric Rhizomes

5.2.1 Sample Preparation

The quality matured dried turmeric rhizomes as available in the local market of Haldia (West Bengal, India) were purchased, checked carefully to separate any other impurity (if present) and dried under controlled condition of 35^0C in a laboratory drying unit (Fig 4.11) having air evacuated system as described in section 4.9.1 for 12 h. This moisture treatment step was carried out to minimize the moisture content of the turmeric sample below 12% since the moisture level

above this value hinders the rate of mass transfer and solubility of the volatile matter in the solvent CO_2 used for SFE [Ivanovic, 2011].

The moisture treated turmeric rhizomes were then milled into smaller size particles in the mixture grinder (Philips Mixer Grinder HL7720) and classified into three fractions with the help of a sieve shaker arrangement assembled with 16 - 80 mesh sieves from Tyler standard screen series. The average particle sizes (D_P) of different fractions were determined following the mass mean diameter calculation. The ground turmeric samples of different sizes were stored separately after packaging in air-tight polyethylene bags for later use in SCO_2E experiments. Cold and dark place was chosen to store the turmeric sample since curcuminoids, the natural pigment of turmeric, degrade in contact with light, heat, and oxidative conditions [Buescher, 2000].

These preprocessing steps of dried turmeric rhizomes buds are shown in Fig. 5.2.

Fig. 5.2: Preprocessing Steps of Turmeric Rhizomes before SCO_2E

5.2.2 Moisture Content

The moisture content of both the ground raw turmeric rhizomes and moisture treated turmeric rhizomes were measured as described in section 4.9.1 using the "SARTORIUS MA45C" moisture analyzer (Fig. 4.11). The moisture analysis results were prepared by averaging the results obtained from triplicate measurements.

5.2.3 Determination of Total Amount of Extractable Oil (Global Yield)

In the present study, the amount of extractable oil or global yield present in the sample of turmeric rhizomes was determined with the help of traditional solvent extraction method using a

Soxhlet apparatus. To perform the experiment approximately (30 ± 0.1)gm of comminuted turmeric sample (from the feedstock having 0.6mm average particle size that was intended to use in SCO_2E studies) was wrapped in a Whatman filter paper thimble and placed inside the main extraction chamber of the Soxhlet apparatus. The bottom end of the Soxhlet extractor was connected with round bottom reflux flask of 500 mL capacity and the upper end was attached with the condenser system as described in section 5.1.3. A volume of200mL of solvent n-hexane was taken then in the distillation flask. Then placing the round bottom flask over the heating mantle as shown in Fig. 4.10, heating of the extraction solvent was started and water circulation through the condenser system was also allowed simultaneously. The extraction was carried out for 8 hrs to ensure complete separation of soluble matters.

The extract of turmeric oil was concentrated by evaporating solvent at 50^0C with the help of a rotary vacuum evaporator. Then residual oil temperature was allowed to drop at room temperature and weighed using precision balance (Citizen CTG 602). The reported value of global yield is the mean of three replications.

The oil recovery was expressed as percentage oil yield with respect to the mass of solid feed used for extraction by using Eq. (4.1).

5.2.4 SCO_2E of Turmeric Powder using Various Geometric Configurations

The performances of the two types of feed bed geometry, namely, conventional cylindrical feed bed (B1) and annulus feed beds (B2 and B3), to extract essential oil from turmeric rhizomes with the help of SCO_2E experiments were evaluated and compared to establish which type of bed geometry influences the extraction of turmeric oil more. SCO_2 extraction experiments of ground turmeric powder were carried out applying the same extraction temperature and pressure for the same extraction time (t_E) of 240 minutes varying only the bed geometry of extraction vessel by introducing external feed bed B1 or B2 or B3. The samples were collected at intervals of 15, 30, 45, 60, 90, 120, 150, 180, 210, & 240 minutes respectively using separate sampling bottles, weighed separately using precision balance (Citizen CTG 602) and recorded to construct OECs. Other parameters of extraction like the mass of feed, particle size, solvent flow rate, and separators conditions were kept constant. The extraction procedure using SCO_2 as solvent was followed as per section 4.5. All the experimental data such as mass of feed (F), particle size (D_P), solvent flow rate (Q_{CO2}), extraction pressure (P in E), extraction temperature (T in E), separators operating conditions, initial static period of extraction (t_S), period of extraction (t_E) all are provided in Table-5.6. The yield was expressed as %OY following Eq. (5.1). All the assays were replicated twice for double sanguine. Finally, the OECs for all these three-bed geometries were plotted and compared.

Table-5.6: Experimental Data for the Extractor Performance Study

F (gm)	D_P (mm)	Q_{CO2} (gm/min)	T in E (^{0}C)	P in E (MPa)
500	0.3	10	50	24.5

T in S-I(^{0}C)	P in S-I (MPa)	T in S-II(^{0}C)	P in S-II(MPa)	t_s (min)	t_E (min)
30	≈ 6	25	≈ 5	20	240

Here F - Mass of feed, D_p – Particle size, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, E – Extractor, S-I - Separator I, S-II – Separator II, t_s - Static period of extraction, t_E – Extraction time

5.2.5 Experimental Design to Optimize SCO_2E

The process of turmeric oil extraction was optimized in the present study choosing some important factors which influence the yield quantity and quality obtained from the SCO_2E. The role of these process variables on extraction may be direct/indirect, also independent/interactive in nature [Montgomery, 2001]. For optimization, among the three feed bed extractors which one extracted turmeric oil most efficiently using solvent SCO_2 in the previous study (Section 5.2.4), aiming performance evaluation of B1, B2, and B3, was selected as the optimal geometric condition within this design boundary. Using this optimal bed geometric configuration for the purpose of feed loading during SCO_2 extraction experiments three factors which were chosen to optimize the extraction process to maximize the yield of turmeric oil in this study are (i) pressure (A1), (ii) temperature (A2), and (iii) particle size (A3). The yield was expressed as %OY following Eq. (5.1). Thus in this optimization process yield of oil was the response variable that would be expressed as a function of these three input variables, A1, A2, and A3.

Once the process variables and response variable were selected, the maximum and minimum levels of the process variables that would be controlled during optimization were defined to build up the experimental design. The methodology chosen from various standard techniques developed for the experimental design and data analysis was statistical analysis based RSM. The experimental design of these three input variables was build-up following the FC-CCD technique developed under RSM as it helps to plan a limited number of experiments within the boundaries of the variables in such a way that would direct towards valid statistical inferences. Following the ways to carry out FC-CCD, at first three levels of the above mentioned three input variables (A1, A2, and A3) were provided with appropriate coding as (−1), (0) and (+1) in Table-5.7.

Table-5.7: Three levels of selected variables chosen for FC-CCD

Pressure, A1 (MPa)	Temperature, A2 (^{0}C)	Particle size, A3 (mm)
21.6 (-1)	40 (-1)	0.3 (-1)
24.5 (0)	50 (0)	0.6 (0)
27.5 (+1)	60(+1)	0.9 (+1)

From Table-5.7 it is clear that the levels of temperature and pressure as selected for SFE studies of turmeric oil were 40^{0}C (-1), 50^{0}C (0), and 60^{0}C (+1) and 21.6 MPa (-1), 24.5 MPa (0), and 27.5 MPa (+1), respectively. Temperature levels were selected considering some factors – (i) Separation of turmeric oil is not simple like clove oil, (ii) some literatures indicate positive impact of temperature on turmeric oil yield [Chang, 2006; Priyanka, 2018], and (iii) high temperature above 60^{0}C may cause quality deterioration due to thermal degradation of heat-sensitive components. In selecting pressure levels, highest pressure of 27.5MPa was chosen considering the design pressure of the extractor (29.42 MPa) and lowest pressure of 21.6 MPa was selected considering pressure-density-solvent-solubility proportional relation and the favorable pressure value as recommended for turmeric oil extraction (at least 26MPa) [Chang, 2006]. Two particle sizes were chosen above the size 0.45mm, that was recommended as optimum in some previous works, [Chang, 2006; Priyanka, 2018] to inspect the influence of modified bed geometry to overcome the negative impact of larger particle size.

During SCO_2E experiments of turmeric rhizome sample, all other important parameters such as mass of feed loaded (F), solvent flow rate (Q_{CO2}), initial static period of extraction (t_S), extraction time (t_E), separators temperature and pressure were kept constant. These data are provided in Table-5.8. The ground turmeric sample used in different runs was taken from the same sample preprocessed and stored previously.

Table-5.8: Experimental Data for the Extractor Performance Study

F (gm)	Q_{CO2} (gm/min)	T in S-I(^{0}C)	P in S-I (MPa)	T in S-II(^{0}C)	P in S-II(MPa)	t_s (min)	t_E (min)
500	18.5	30	≈ 6	25	≈ 5	20	240

Here F - Mass of feed, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, S-I - Separator I, S-II – Separator II, t_s - Static period of extraction, t_E – Extraction time

For the selected three controlling factors, FC-CCD produced a table consisting of twenty sets of experimental conditions to conduct twenty SCO_2E tests. From this stage of statistical analysis, the help of some software packages was needed to handle such multi-variable complex processes. In the present study Design Expert -11 was used to perform the necessary steps of optimization. The

FC-CCD data set of three independent variables with their coded levels as generated in the design layout is given in Table-5.9.

Table-5.9: FC-CCD data of three factors to conduct SCO_2E of Turmeric rhizome

Run No.	Input Factors		
	Pressure, A1 (MPa)	Temperature, A2, (^{0}C)	Particle Size, A3 (mm)
1	24.5 (0)	40(-1)	0.6(0)
2	21.6 (-1)	40(-1)	0.3(-1)
3	24.5 (0)	50(0)	0.6(0)
4	24.5 (0)	50(0)	0.6(0)
5	27.5 (+1)	50(0)	0.6(0)
6	24.5 (0)	50(0)	0.6(0)
7	24.5 (0)	50(0)	0.6(0)
8	21.6 (-1)	60(+1)	0.3(-1)
9	27.5 (+1)	60(+1)	0.9(+1)
10	21.6 (-1)	60(+1)	0.9(+1)
11	27.5 (+1)	60(+1)	0.3(-1)
12	24.5 (0)	50(0)	0.6(0)
13	27.5 (+1)	40(-1)	0.9(+1)
14	27.5 (+1)	40(-1)	0.3(-1)
15	24.5 (0)	50(0)	0.3(-1)
16	21.6 (-1)	50(0)	0.6(0)
17	24.5 (0)	50(0)	0.9(+1)
18	24.5 (0)	60(+1)	0.6(0)
19	21.6 (-1)	40(-1)	0.9(+1)
20	24.5 (0)	50(0)	0.6(0)

Once all the SCO_2E experiments using turmeric sample were carried out following FC-CCD design, the yield of oil as %OY were entered in appropriate response column of design expert's

design layout. Then the responses were analyzed using RSM as explained in details in section-5.1.4. Thus the response variable was fitted with the input factors by statistically significant mathematical model applying the analysis of variance (ANOVA). The influence of each independent factor and their interactions were examined and estimated statistically during ANOVA, which are explained by graphical means in the result section.

5.2.6 Chemical Characterization of Turmeric Oil

The compositions of aromatic bioactive ingredients present in the turmeric oil samples were identified with the help of an advanced standard gas chromatograph-mass spectrometer, GCMS-QP2010 SE (SHIMADZU, Kyoto, Japan) in the quality control laboratory of Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India. The detail information of GCMS operational procedure, sample preparation manual, and inbuilt GCMS solution software with MS library was discussed in section 4.9.2.

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Chapter 6

BIOEFFICACY TESTS OF CLOVE ESSENTIAL OIL

6. Introduction

All cures can be found in nature, therefore to find a natural cure we must look for it in nature. Synthetic antioxidants are used for several purposes, such as dietary supplements, food preservatives, antiageing ingredient in cosmetics [Sherwin, 1990; Andreassi, 2004; Poljsak, 2013]. Different research works have been suspected that inappropriate dosing of most commonly used synthetic antioxidants cannot provide complete protection against oxygen free radicals attack in the body due to their carcinogenic and toxic nature [Dundar, 2000; Halliwell 2007; Poljsak, 2012]. Therefore, isolation of antioxidant property bearing phenolic components from natural sources and their utilization in a controlled way in absorbing free radicals without altering the endogenous antioxidative defense system is demanding more and more attention of the researchers [Duh, 1998; Song, 2010]. In this study, clove oil obtained from SCO_2E process was tested to evaluate antioxidant properties of clove oil.

6.1 Antioxidant Activity Study applying Spectrophotometric Methods

Several numbers of spectrometric techniques have been proposed to evaluate the antioxidant capacity. These methods of total antioxidant capacity assessment use either radicals or metal ions as the oxidizing agents which react with the antioxidant molecules. The chemical reactions involve either hydrogen atom transfer (HAT) or single electron transfer (SET) between ROS and antioxidant molecule to form a stable compound in the determination of antioxidant capacity assays [Huang, (2005); Prior, (2005)]. In this reduction reaction, the oxidants colour change and stability increases. The degree of colour change is measured spectrophotometrically at a given wavelength and correlated with the antioxidant concentration or capacity [Zulueta, 2009]. HAT reactions are generally independent of solvent and pH and faster (a few seconds to minutes) [Zulueta, 2009]. On the other hand, SET reactions are pH-dependent, relatively slow, and solvent dependent as compared to HAT [Karadag, 2009].

Extracts of clove buds were reported to show amazing antioxidant properties in different works [Chaieb, 2007; Gülçin, 2012; Ivanovic, 2013; Politeo, 2006]. Their antioxidant properties may be assessed in terms of the presence of phenol content, their free radical scavenging effect, metal chelating activity, total antioxidant property, reducing power, lipid peroxidation inhibition, etc. In this study, clove oil was extracted by SCO_2E method using an extraction vessel of annulus geometry that was different from the conventional cylindrical type. Thus the extract from this newly designed extractor was tested to determine biomedicinal properties.

In this study, different antioxidant assays were determined such as total phenolic content (TPC) using folin-ciocalteu reagent (FCR), free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), metal chelating capacity (Fe^{2+} ions chelating assay), potassium ferricyanide reducing power, and lipid peroxidation inhibition assay. Experimental methods followed to determine various antioxidant assays mentioned above are described below.

6.1.1 Total Phenolic Content (TPC) using Folin-Ciocalteu Reagent (FCR)

The Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenol content of the clove extract using Gallic acid as a standard as described by Singleton and Rossi (1965) and McDonald, (2001) with slight modification [McDonald, 2001]. Since FCR method is based on

oxidation/reduction reaction following SET mechanism, it is considered to quantify the antioxidant capacity of plant extracts [Prior, 2005]. It involves the reaction of phenolic content with phosphomolybdate and phosphotungstate (yellow colour) and reduces them to form blue colour complexes, hexavalent phosphomolybdic, and phosphotungstic complexes.

The Folin-Ciocalteu spectrophotometric experimental procedure followed in this study was as described by McDonald, (2001) with minor modification. 100 µl of clove oil obtained from SCO_2E (operated at the optimum condition) was added with 900 µl of double-distilled water. It was mixed with 1500 µl of 1N Folin-Ciocalteu reagent (a mixture of sodium molybdate, sodium tungstate, and other reagents). This solution was strongly shaken and kept to dark for 5 min. Then, 4 ml of 20 % aqueous sodium carbonate (Na_2CO_3) was added and the mixture was kept to a dark place for 30 min at room temperature before the colorimetric study of the end blue colour complex solution. The absorbance of this developed colour was recorded at 738 nm using an UV-Spectrophotometer (Shimadzu UV-vis 1800 Spectrophotometer) and was expressed as milligrams of Gallic acid equivalents (GAE) per gram of clove extract. The calibration curve of Gallic acid was prepared using Gallic acid solutions (in methanol-water solution, 50:50 (v/v)) of seven different concentrations ranging from 1µg/ml to 12µg/ml. All tests were performed in triplicate.

6.1.2 Free radical scavenging activity or DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhy) - scavenging activity

The DPPH$^{\bullet}$ method is one of the most popular protocols frequently used to investigate the free radical-scavenging capacity of plant extracts developed by Brand-Williams (1995), Yokozawa (1998), Gülçin (2011), and Ivanovica,(2013). In the present study, the method of Blois, previously described by Gülçin (2011), was used with slight modifications to assess the DPPH_ free radical–scavenging capacity of eugenol. In the presence of phenolic free radical-scavenging antioxidants, hydrogen atoms are transferred to the DPPH radicals that are neutralized in the process (Fig. 6.1). As a result, deep purple colour (detected at 517nm) of DPPH radicals transform into pale yellow or colourless hydrazine. Reduced absorption intensity is used as a measure of free radical scavenging activity of plant extract.

For the experiment, a solution of DPPH$^{\bullet}$ in ethanol (0.1 mM) was prepared. A constant volume (0.5 ml) of DPPH$^{\bullet}$ solution was added to tubes containing different concentrations of Clove essential oil (0 – 40µg/ml) prepared by diluting pure clove oil in ethanol. Then shaking the mixtures vigorously, the micro test tubes were allowed to stand at room temperature for 30 minutes in the dark. Absorbances of the mixtures were measured spectrometrically at 517 nm against blank samples. Gallic acid was used as standard antioxidant. The decrease in absorbance is attributed to decolourisation of more DPPH$^{\bullet}$ due to scavenging of available radicals by phenolic contents and percentage decrease in absorbance with respect to control is expressed as percentage scavenging by the extract. The antioxidant property of extract was quantified by their EC_{50} value that was equivalent to the concentration of clove oil necessary to obtain 50% scavenging effect or reduce 50% of DPPH$^{\bullet}$ present in the solution. EC_{50} value was calculated using the regression equation generated from the plot of % scavenging vs. concentration of clove oil sample. All determinations were carried out three times.

$$\%\ Inhibition\ Activity\ or\ \%\ Scavenging = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100$$

---------- (6.1)

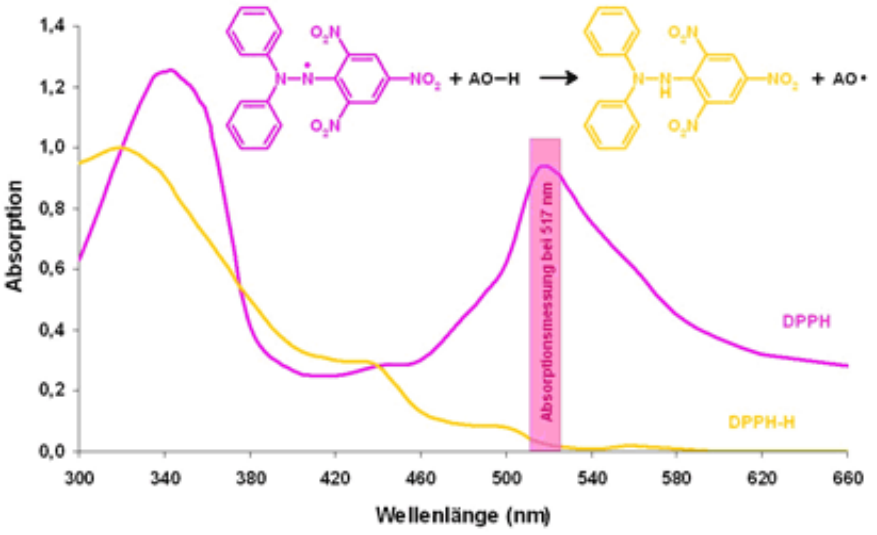

Fig. 6.1: DPPH$^{\bullet}$ neutralization reaction with phenolic Antioxidant compound (AO-H)
[https://www.researchgate.net/post/DPPH_Antioxidant_assay_Method_Development2]

6.1.3 Metal Chelating Capacity or Fe^{2+} Ions Chelating Assay (FICA)

In animal and human body transition metal ions in their higher oxidation state are capable of starting chain reactions with free radicals giving rise to the voluminous amount of free radicals being generated (due to the conversion of $O_2^{-\bullet}$ and H_2O_2 into $HO^{\bullet}$). These reactions are known as Fenton reactions [Quideau, 2011]. Antioxidants like phenolic content of plant extract can prevent the Fenton reactions by forming a chelate with the metal ions. Among various transition metals, iron is highly reactive and known as the most important lipid oxidation pro-oxidant [Halliwell, 1984]. The rate of free radicals production by Ferric ions ($Fe^{3+)}$ is tenfold lesser than that of ferrous (Fe^{2+}) ions [Gülçin, 2012]. Therefore, determination of ferrous ions metal chelating capacity of antioxidant property bearing phenolic content of clove is important.

The metal chelating capacity of Clove essential oil was measured following the method of Dinis and co-workers (1994). Briefly 0.2 ml (2 mM) ferrous chloride ($FeCl_2$) solution was added to different concentrations (0-100 µg/ml) of clove essential oil diluted with methanol. For the initiation of the chelation reaction, 0.4 ml ferrozine iron reagent (5 mM) was added to the reaction mixture. The micro test tube containing the reaction mixture was shaken vigorously and kept at room temperature for incubation for 15 minutes to reach reaction equilibrium. In the medium phenolic content of clove oil and ferrozine compete with each other to chelate with Fe^{2+}. Ferrozine forms a red coloured complex. In the presence of other antioxidant ferrozine-Fe^{2+} complex formation was reduced and resulted in a decrease of intensity of the red colour [Dinis, 1994]. The color reduction was then measured spectrophotometrically at 562 nm. The control experiment was performed using $FeCl_2$ and ferrozine reagent.

6.1.4 Potassium Ferricyanide Reducing Power or Fe^{3+} Reducing Antioxidant Power (FRAP)

The ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of clove essential oil was measured following the methodology proposed by Oyaizu (1986). In this method, antioxidant power of clove oil was utilized to reduce the transition metal ion in the form of ferricyanide complex to its lower oxidation ferrous (Fe^{2+}) state. This reduction reaction is redox type that involves the transfer of electrons between easily reduced oxidant (Fe^{3+}) and reductants antioxidant molecules (Benzie and Strain, 1996). In brief clove extract of different concentrations ranging from (0- 100 µg/ml) were added with phosphate buffer (0.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 ml, 1%). The role of phosphate buffer in this reaction was maintaining pH at 6.7, which is the pH of the human body. The mixtures were then allowed for incubation at 37°C for one hour. 10 % Trichloroacetic acid (0.5 ml) was added to the mixtures to stop further reduction of ferric ions to ferrous by any other means. It was then centrifuged for 10 min at 3000 rpm. 0.2 ml of this solution from the upper layer was mixed with $FeCl_3$ (0.2 ml, 0.1 %). The potassium ferrocyanide further reacted with $FeCl_3$ to form an intense prussian blue complex. The absorbance of this coloured solution was detected at 700 nm in the spectrophotometer (Shimadzu UV-vis 1800 Spectrophotometer). Negative control was prepared by adding all the items except clove oil. The volume of the clove oil was substituted by PB buffer in the control set. Increased absorbance of the reaction solution indicates more reduction of ferric to ferrous form and an increase of ferric ions reduction capability. Gallic acid was taken as a positive control.

6.1.5 Lipid Peroxidation Inhibition Assay (LPIA)

Damage of the body cells would only be initiated when free radical oxidizes the cell membrane and enters the cell. This cell membrane is made of lipid. Thereby, it becomes important to check whether the antioxidant is able to prevent lipid peroxidation and its perforation by free radicals.

Thiobarbituric acid-reactive species (TBARS) assay as described by Dutta et al. (2011) with minor modification was followed to quantify Lipid Peroxidation Inhibition effect of the clove buds extract using goat's erythrocytes. Goat's erythrocytes were collected from butcher's shop at the local market of Haldia. Blood was then allowed for centrifugation at 3000 rpm for 10 minutes to separate plasma. Collected erythrocytes were dissolved in chilled phosphate buffer saline (pH 7.4) to make 10 % solution. Different concentrations of clove essential oil (0-100 ug/ml) and blood sample were taken in screw-capped tubes. $FeSO_4$ (0.07 M, 0.3 ml) was mixed to each test tubes for initiation of peroxidation and incubated for 30 min at 37 °C. After that, 20 % acetic acid (1.5 ml), 0.8 % thiobarbituric acid (TBA) in 1.1 % SDS (1.5 ml) and ice-chilled 20 % trichloroacetic acid (TCA) (500 ul) were added carefully to all the test tubes and were gently vortexes. The reaction mixtures were heated at 95 °C for 1 h in a boiling water bath. After the incubation period, all the samples were cooled to room temperature; 5 ml of butan-1-ol were added to each tube. All the samples were then centrifuged at 3000 rpm for 10 min. The organic layer of the reaction mixture was tested spectrophotometrically to measure the absorbance at 550 nm.

Statistical analysis

All experiments were verified in triplicate. Data points were reported by the mean of the measured values. Statistical analysis was carried out using MS-Excel software. Gallic acid or BHT was considered as standard antioxidant.

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Chapter 7

RESULTS AND DISCUSSION

7. Introduction

This chapter covers results of the present study to find out the effect of some critical parameters along with the geometry of the extraction vessel that affect the quantity and quality of natural antioxidant enriched medicinal plant essential oils extracted with the help of SCO_2 solvent. The results of antioxidant properties of extracted oil were also reported as these properties provide information of their medicinal benefits. The obtained results are arranged sequentially as experiments or testing were done and discussed in the following subsections:

I. Optimization of SCO_2 extraction of clove buds to obtain valuable essential oil and parametric studies to assess the effects of various parameters within the design boundaries.
II. Determination of kinetic parameters of clove oil extraction process from their OECs.
III. Determination of chemical constituents of Clove essential oil obtained from SCO_2E.
IV. Optimization of SCO_2 extraction of turmeric rhizomes to obtain valuable essential oil and parametric studies to assess the effects of various parameters within the design boundaries.
V. Determination of chemical constituents of turmeric essential oil obtained from SCO_2E.
VI. Evaluation of antioxidant properties of clove oil obtained from SCO_2E.

7.1 Outcomes of Clove Oil Extraction Experiments

7.1.1 Moisture Content, Particle Size, and Global Yield

The moisture content of the raw milled clove sample was 13.25%. The moisture content of plant materials affects the oil yield of extraction and selectivity of certain bioactive compounds in SCO_2. As mentioned previously it was reported that the influence of moisture content on the rate of mass transfer and on the dissolving power of oil molecules in SCO_2 has no hindrances if it lies in the range from 3% to 12% [Ivanovic, 2011]. The main problem faced with high moisture content in the large scale operation is the co-extraction of water and its significant presence in the extract obtained from the outlet of the separator. Information from literature says that in the case of clove buds after 100 minutes of extraction period water comes out along with oil from the separators outlets [Prado, 2011]. For lab scale study where very less amounts of samples have handled, the presence of water may not exist or insignificant [Berna, 2000]. With increasing process scale, the existence of water is not ignorable. These water-oil mix yields create severe problems in developing the pure yield data w.r.t. time scale required for plotting OECs. For minimization of the water co-extract, the clove buds sample was dried before the extraction experiments were carried out. The moisture content of the dried milled clove sample was 6.83%.

This preprocessed clove sample was classified into three different particle sizes of 1.5mm, 1mm, and 0.64mm that were used later in the extraction experiments. The total extractable matter or the global yield of clove buds that were evaluated by solvent extraction using Soxhlet Apparatus to use as reference yield in SFE study was 19%. The result of the solvent extraction of cloves was found to agree well with the available literature [Gopalakrishnan, 1988; Nurdjannah, 2012; Zabot, 2014]. All these results are provided in Table-7.1.

Table-7.1: Moisture Content, Particle Size and Global yield of Clove Sample

Moisture content of raw Clove Buds (wt %)	Moisture content of dry milled Clove Powder (wt %)	Particle Size			Global Yield of Clove Oil (%OY)
		DP1(mm)	DP2 (mm)	DP3 (mm)	
13.25	6.83	0.64	1	1.5	19

7.1.2 Optimization of SCO_2 Extraction Process for Extraction of Clove Buds Essential Oil

All the SCO_2 extraction experiments of dried milled clove bud sample were carried out in the semi-continuous supercritical fluid extraction equipment (Model No: CSL/SCF/1L2/400). The experimental conditions of the selected factors towards optimization of the SFE process were designed using face-centered central composite design (FC-CCD) pattern developed under statistical analysis technique of response surface methodology (RSM). Three factors, namely ARSEF (X1) related to bed geometry, temperature (X2), and pressure (X3), were examined in this optimization study at their three levels. Same pre-processed feed in equal quantity was used in all the runs. The oil samples of each experiment were collected following the same periodic interval pattern as described in section 5.1.5 to study the effect of all selected parameters on the supercritical fluid extraction rate.

The responses of FC-CCD in this analysis were the yields of clove oil expressed as %OY (Eq. 2) obtained from the SFE experiments conducted following the different combinations of three process variables (X1, X2, and X3) as per FC-CCD design criteria (Table-5.3). All the response data, along with their input factors, are shown in Table-7.2.

In order to fit the response data with selected factors of extraction by a suitable model, all the data were analysed for linear, two-factor interaction (2FI) and quadratic models based on R^2, standard deviation, adjusted R^2, predicted R^2, "PRESS" value, F-values, p-values, and lack-of-fit tests results. The quadratic model produced highest F-value (205.23) and lower p-value < 0.0001, i.e., the quadratic model indicated an insignificant lack of fit for all the input and response data. This model came out as a suggested model for exhibiting low value of standard deviation (0.0854), high value of R^2 (0.9978), lowest value of "PRESS" (1.13) and maximized adjusted R^2 (0.9958) and predicted R^2 (0.9659) [having a difference less than 0.2] among all types models mentioned here.

The statistical significance of each term of the quadratic model was evaluated by performing ANOVA test. The results of ANOVA for the suggested model were illustrated in Table-7.3. High Model F-value of 503.32 indicates the response surface quadratic model is significant. P-values of all individual terms of the quadratic model equation less than 0.0500 reflect the importance of all the model terms. Thus ANOVA test provides the valuable information about the dependency of clove oil yield (%OY) on all the selected factors of bed geometry of the extraction vessel (X1), extraction temperature (X2) and extraction pressure (X3) from various angles.

Table-7.2: FC-CCD data of three independent variables with their coded levels and response as percentage oil yield (% OY) of clove buds using SCO_2 Extraction

Run No.	Input Factors			Response
	$ARSEF, X1 = \frac{(r_o - r_i)}{2L}$	Temperature, X_2 (^{0}C)	Pressure,X_3 (MPa)	%OY (gm Oil/ 100 gm Feed)
1	0.0283 (0)	45 (+1)	19.6 (0)	16.42
2	0.0238 (-1)	35 (-1)	24.5 (+1)	15.92
3	0.0283 (0)	40 (0)	19.6 (0)	16.07
4	0.0283 (0)	40 (0)	24.5 (+1)	16.83
5	0.0327 (+1)	35 (-1)	24.5 (+1)	13.51
6	0.0238 (-1)	45 (+1)	24.5 (+1)	18.05
7	0.0327 (+1)	40 (0)	19.6 (0)	14.55
8	0.0283 (0)	40 (0)	19.6 (0)	16.07
9	0.0283 (0)	40 (0)	19.6 (0)	16.07
10	0.0238 (-1)	40 (0)	19.6 (0)	16.81
11	0.0327 (+1)	45 (+1)	14.7 (-1)	14.13
12	0.0283 (0)	40 (0)	19.6 (0)	16.07
13	0.0283 (0)	40 (0)	14.7 (-1)	15.03
14	0.0238 (-1)	35 (-1)	14.7 (-1)	14.52
15	0.0238 (-1)	45 (+1)	14.7 (-1)	15.79
16	0.0283 (0)	35 (-1)	19.6 (0)	14.74
17	0.0327 (+1)	45 (+1)	24.5 (+1)	15.85
18	0.0327 (+1)	35 (-1)	14.7 (-1)	12.11
19	0.0283 (0)	40 (0)	19.6 (0)	16.07
20	0.0283 (0)	40 (0)	19.6 (0)	16.07

Here X_1, ARSEF; X_2, Extraction Temperature; X_3, Extraction Pressure; %OY, Percent Oil Yield of Clove Buds

Table-7.3: ANOVA for the suggested model to analyse parametric effects on clove oil yield (% OY)

Source	Actual Regression Coefficients	Sum of Squares	df	Mean Square	F-value	p-value
Model		33.06	9	3.67	503.32	< 0.0001
X_1, ARSEF	779.2781	11.97	1	11.97	1639.87	< 0.0001
X_2, Temperature	1.56208	8.91	1	8.91	1221.01	< 0.0001
X_3, Pressure	0.288898	7.36	1	7.36	1008.67	< 0.0001
$X_1 X_2$	5.39326	0.1152	1	0.1152	15.78	0.0026
$X_1 X_3$	-3.09562	0.0365	1	0.0365	4.99	0.0494
$X_2 X_3$	0.00602	0.174	1	0.174	23.85	0.0006
X_1^2	-20888.088	0.4705	1	0.4705	64.47	< 0.0001
X_2^2	-0.020545	0.7255	1	0.7255	99.41	< 0.0001
X_3^2	-0.006815	0.0736	1	0.0736	10.09	0.0099
Residual		0.073	10	0.0073		
Lack of Fit		0.073	5	0.0146		
Pure Error		0	5	0		
Cor Total		33.13	19			
Std. Dev.	0.0854					
R^2	0.9978					
Adjusted R^2	0.9958					
Predicted R^2	0.9659					
Adeq Precision	95.8806					

Here X_1, X_2, and X_3 relate the effects of main process variables ARSEF (r_o-r_i/2L), temperature (°C), and pressure (MPa), respectively, on the response (%OY). X_1^2, X_2^2, and X_3^2 represent the quadratic effects of the same input variables. X_1X_2, X_1X_3, and X_2X_3show the interactive effects of three possible combinations of three variables (i) ARSEF and temperature; (ii) ARSEF and pressure, and (iii) temperature and pressure, respectively.

7.1.2.1 Model Equation obtained from RSM

The final equation for the predictive quadratic model in terms of actual factors within the region under investigation can be expressed in a generalised way by the following formula:

$$\%OY = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X3 + \beta_{12} X1X2 + \beta_{13} X1X3 + \beta_{23} X2X3 + \beta_{11} X1^2 + \beta_{22} X2^2 + \beta_{33} X3^2 \quad \text{-------(7.1)}$$

where %OY is the actual response;

X1, X2, and X3 are the un-coded variables ARSEF, extraction temperature, and pressure, respectively;

β_0 is the regression coefficient of intercept;

β_1, β_2, and β_3 are the regression coefficients for the linear fit of individual factor;

β_{12}, β_{13}, and β_{23} are the regression coefficients for FI fit (i.e., ARSEF-temperature, ARSEF- pressure, and temperature-pressure interactive factors); and

β_{11}, β_{22}, and β_{33} are the regression coefficients for quadratic fit.

The evaluated values of these regression coefficients from the ANOVA test to fit the yield of clove oil obtained from SFE experiments following FC-CCD design conditions of three input factors are given in Table-7.4.

Table-7.4: Coefficients of Predictive Quadratic Model obtained from ANOVA for Clove

	β_0	β_1 (10^2)	β_2	β_3	β_{12}	β_{13}	β_{23}(10^{-3})	β_{11}² (10^3)	β_{22} ² (10^{-3})	β_{33} ² (10^{-3})
%OY	-31.02006	7.793	1.562	0.289	5.393	– 3.096	6.02	-20.888	-20.545	-6.815

This equation in terms of actual factors with evaluated regression coefficients is useful in making predictions about the response, yield (%OY) of clove buds SFE, for given levels of each input factor. Here, the value of each factor within the specified level should be substituted in their original units.

Predicted responses calculated using final quadratic equation (7.1) for twenty runs performed based on FC-CCD and their actual responses are provided in Table-7.5 (a) & (b). These results of the predicted response of ANOVA vs. actual response in terms of %OY are presented graphically in Fig. 7.1.

Table-7.5(a): Actual Value of %OY and Predicted Value of %OY as per ANOVA

Run Order	1	2	3	4	5	6	7	8	9	10
Actual Value of %OY	16.07	16.81	12.11	16.07	16.07	14.13	16.83	15.92	15.03	16.07
Predicted Value of %OY	16.08	16.76	12.19	16.08	16.08	14.02	16.77	16.04	15.06	16.08

Table-7.5(b): Actual Value of %OY and Predicted Value of %OY as per ANOVA

Run Order	11	12	13	14	15	16	17	18	19	20
Actual Value of %OY	13.51	14.52	16.07	18.05	15.85	15.79	14.55	16.07	14.74	16.42
Predicted Value of %OY	13.47	14.48	16.08	17.98	15.9	15.83	14.57	16.08	14.62	16.51

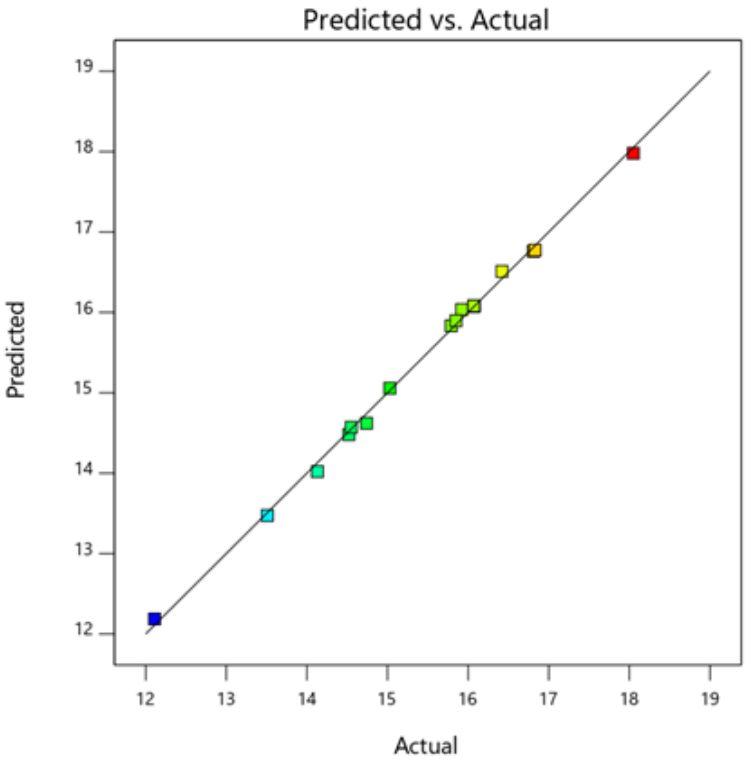


Fig 7.1: Predicted Response of %OY vs. Actual Response of %OY for Clove

7.1.2.2 Effects of Extractor Bed Geometry, Pressure and Temperature on Clove Oil Yield

The influence of individual parameter chosen in this optimization study on the yield of extraction of clove buds can be explained with the help of perturbation plot (Fig. 7.2). Perturbation plot is a graphical representation in a single response plot of all factors showing the variation of response with varying individual input factor over its range while all the other factors are kept constant. It also helps in finding the relative significance of the factor from the nature of curvature. If the slope or curvature for a particular factor is steeper than others, then it indicates the higher sensitivity of the response to that factor. A relatively flat curvature on the other side indicates insensitivity of the response of RSM design towards the variation of that particular input factor. For multiple factors, the perturbation plot is sometimes beneficial to find the most influential factors for the response.

❖ Influence of Extractor Bed Geometry

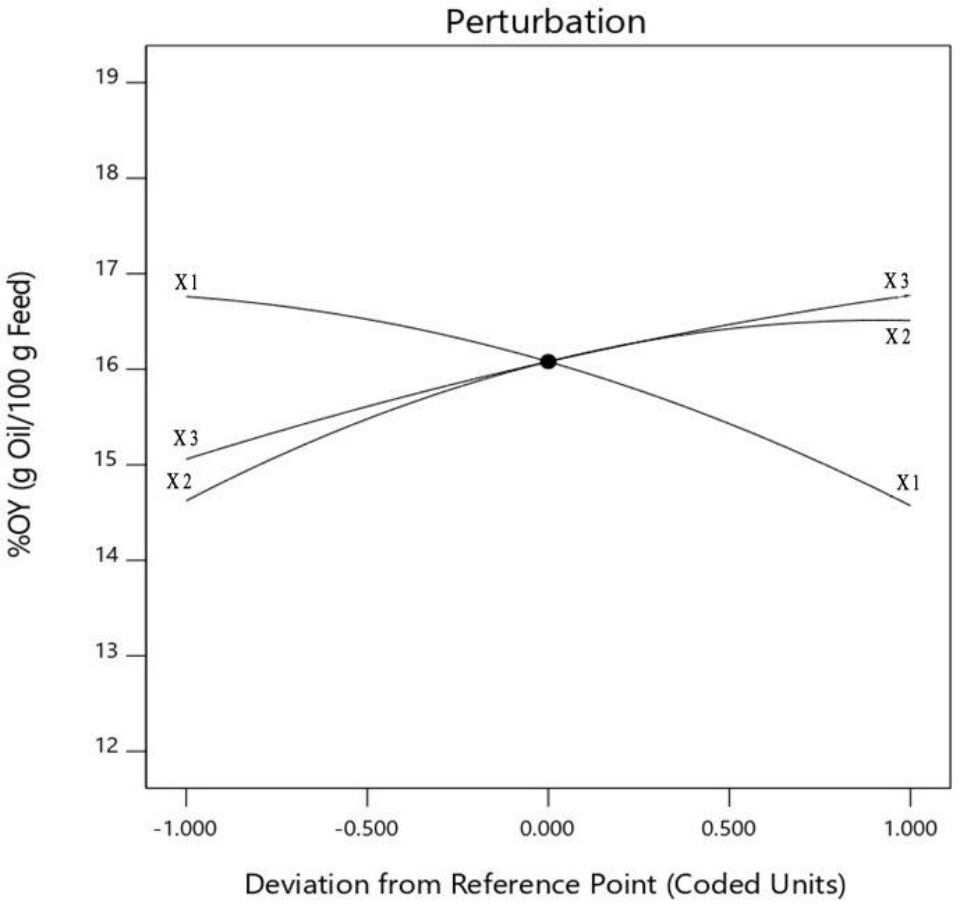
The plot of %OY vs. factor X1 in the perturbation plot (Fig. 7.2) represents the influence of the geometry of the extraction vessel of SFE unit on the yield of clove oil. In this plot the point (+1) on X-axis indicates the conventional cylindrical extractor vessel (B1) that was designed without any annular channel, point (0) indicates the modified annular extractor bed (B2) that was designed introducing annular channel of smaller diameter (0.75mm) with one blind end in the upstream side and point (-1) on same axis indicates the modified annular extractor bed (B3) that was designed introducing annular channel of larger diameter (1.5mm) with one blind end in the upstream side. Thus this plot is useful to explain the effectiveness of introducing annular geometry over traditional cylindrical geometry inside the extraction vessel.

The steeper slope of %OY vs. factor X1 plot describes the response, %OY of clove oil, is highly sensitive to the bed geometry configurations used in this investigation. As P-values of all linear, FI, and quadratic terms of X1 in ANOVA lie in the range 0.1%-5% (Table-7.3), it suggests that the yield is a strong function of annulus extractor bed geometry. The dimensionless group representing bed geometry, ARSEF, and %OY of clove are found negatively related to each other. It signifies that the yield of clove oil produced from SFE increases gradually and significantly from conventional cylindrical geometry to annular geometry. The highest yield is pointed out in case of annulus extractor vessel with the larger annular channel (B3). Among all FC-CCD design experiments, the maximum yield of 18.05% was obtained using the annular feed bed B3 compared to 16.83% for the annular feed bed B2 and 15.85% for the conventional cylindrical feed bed B1 under all operating conditions as designed by FC-CCD design. These are 83.42% (for B1), 88.58% (for B2) and 95 % (for B3) of reference yield, which was extracted using organic solvent n-hexane in Soxhlet apparatus [Table-7.1].

The results of this study indicated that the use of the annulus bed geometry inside the extraction vessel of SFE unit favours the rate of extraction of essential oil from solid materials compared to regular cylindrical geometry. The kinetic parameters (including the rate of extraction of SFE process for a particular plant material) depend on various factors. Information related with extraction temperature and pressure; co-solvent and properties and nature of botanic matrix when are optimized to improve kinetic parameters another important factor, the geometry of extractor vessel, must be considered since it may alter the system kinetics or shape of OECs. The geometric variation in extractor design greatly influences the extractor's performance by changing the distribution pattern of the solid phase, the tortuous path for solvent flow, mass and heat transfer rates [Zabot, 2012].To retain the kinetic behaviour in extractor of varying geometries, key process parameters which are related with each other are the ratio between the extractor height to the extractor diameter, the ratio of solvent mass to feed mass, etc. [Zabot, 2012]. Similar results were indicated in case of clove buds in the studies of Prado (2011) and Zabot (2014). Rate of mass transfer, hence the oil extraction rate also depends on the contact between the solid sample and solvent. For improving sample-solvent contact and preventing the channelling in the packed bed of extraction vessel, use of stainless steel balls or glass beads were reported in some studies for lab-scale extractor having the volume up to 1000mL. These balls or beads could be mixed with the sample, or the sample was packed in single or multiple layers covering by these balls from both sides [Bimakr, 2012; Yang, 2013; Priyanka, 2018].In this lab-scale study extractor height to

diameter ratio, and solvent mass to feed mass ratio were maintained almost constant, and the extractor feed bed were filled in full capacity with the sample without applying any glass beads or stainless steel balls.

The main change in the extractor design that was proposed in this work is the annulus geometry replacing the conventional cylindrical geometry. This modification in extractor geometry increases the ratio of radial surface area ($2\pi L(r_o + r_i)$) to axial surface area ($\pi(r_o^2 - r_i^2)$) which exhibits a positive impact on the yield of clove buds within its design range. Increasing yield with changing any process parameter without process elongation means a faster rate of mass transfer and enhancement of process kinetics. It is because of the increase of the surface ratio that makes it easier to diffuse the molecules for both the solvent $SCO_2$and solute. This annulus bed geometry also induces turbulence in the fluid phase that raises the convective diffusion. As a result, resistance to mass and heat transfer for oil extraction decreases yielding higher oil mass.



X1: Bed Geometry Factor (m/m), X2: Temperature (ºC), X3: Pressure (MPa)

Fig. 7.2: Perturbation Plot to explain the effects of SFE Optimizing Parameters on %OY of Clove

❖ **Influence of Extraction Pressure**

Extraction pressure or density of SCO_2 solvent is very high-priority determining factor for the SFE as it can control the selectivity and solvation power of the solvent, rate of extraction and hence the quantity and quality of extraction yield [Cherchi, 2001; Liza, 2010].The effect of pressure on the extraction of clove buds essential oil was investigated at three different levels of 14.7MPa, 19.6MPa, and 24.5MPa. The plot of %OY vs. factor X3 in the perturbation plot (Fig. 7.2) shows the role of extraction pressure on yield of clove oil obtained from SFE. The nature of the plot indicates that there is a positive impact of pressure on the oil yield. The extracted amount of oil for the fixed time of extraction increases with increasing pressure throughout the range selected for optimization. As pressure was increased from 14.7MPa to 24.5MPa, the average oil yield also improved from 14.12% to 16.03% (wet basis) for different extraction temperatures and bed geometry. Wenqiang (2007) confirmed a similar effect of pressure in SFE of clove buds in the pressure range 10-30MPa.Yazdani, (2005) also reported an increased yield of cove oil for a rise of pressure from 11MPa to 19MPa using SCO_2 solvent. The application of higher extraction pressure is a key factor for getting increased oil yield. Increased pressure favours the extraction of targeted analytes by improving various properties that play a significant role in solid-fluid extraction, as explained below.

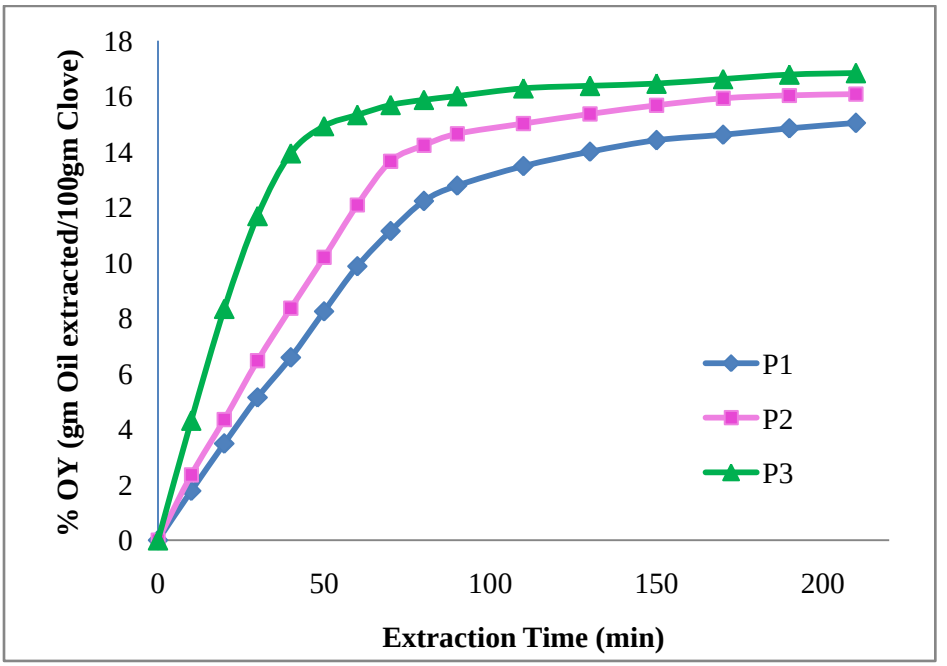
High selectivity of the SCO_2 solvent in the extraction process provides the main advantage of producing concentrated extract and facilitates the high recovery of target analytes [Berglöf, 1999]. Such selective extraction of specific components can be achieved by adjusting the solvation power or solubility of the SCO_2. The solubility (the concentration of oil at equilibrium) is directly proportional to the density and/or polarity of the solvent. Near the critical point of any substance, slight alterations in pressure or temperature result in noticeable changes in the density of the SF, providing opportunities to finely "tune" the properties like solubility, selectivity, mobility of supercritical solvent to a favourable mode. Since the density of solvent SCO_2 and extraction pressure maintain a direct relationship, higher operational pressure of the supercritical extractor attributed to the increase of density of the solvent resulting in the increasing dissolving property [Wenqiang, 2007]. Increasing density reduces the molecular distances between solvent $SCO_2$and solute (oil) molecules and enhances the solvent-solute interaction, raising the solubility of oil in SCO_2, likewise liquid solvents [Liza, 2010]. Hence faster recovery of solute is possible due to the increase of driving force of mass transfer, and it reduces the extraction time for required recovery of oil considerably.

Another key controlling factor in making the rate of mass transfer faster is the diffusivity of the solvent. In the diffusion-based solid-fluid extraction process, solvent CO_2 necessitates diffusing into the solid matrix to dissolve the solute matter and the dissolved matter to diffuse back through the solid mass into the bulk solvent phase. Near the critical point, negligible viscosities of SFs make it easier to penetrate into the solid material. The study of Heidaryan (2011) reported that near 310K temperature viscosity of SCO_2 strongly depends on the pressure. The highest value of viscosity was attained at low temperature and high-pressure region (90MPa), and the minimum viscosity was obtained at low temperature (310K) and low pressure (7.7MPa).Some studies reported an abnormal drop in extraction yield with increasing the pressure level after a particular value [Bimakr, 2012; Liza, 2010; Rezaei, 2000; Wang, 2008]. Rezaei (2000) reported that at a

higher level of pressure the reason of reduction in extraction productivity than that anticipated by the solubility improvement was the reduced rate of diffusion of extractable molecules into the SCO_2 phase. Bimakr (2012) also confirmed the same finding. Xu (2011) pointed out that at high pressure level highly compressed SCO_2 increases the repulsion between the solute and solvent molecules during their interactions. It makes the extraction process more complex for which very high pressure is not always recommended. Pourmortazavi (2007), and Zhang (2010) reported that at an elevated pressure solubility of all solutes of complex matrix increases, producing complex extract and difficulty in the analysis of oil. Consequently, co-extraction of other unwanted solutes can dramatically change the solubility of targeted components.

Hence selective extraction of volatile oil molecules and yield of oil are possible to increase by raising pressure up to the optimum value [Bimakr, 2012].

In the perturbation plot (Fig. 7.2), the oil yield (%OY) and extraction pressure relation appear as almost linear [Xu, 2008]. In the present study, the highest yield of clove oil (18.05%) was obtained at an elevated pressure of 24.5MPa, which was the highest extraction pressure chosen for optimization. Fig. 7.3 is a representation of the amount of oil extracted (%OY) and extraction time for each level of pressure (P1=14.7MPa, P2=19.6MPa, and P3=24.5MPa) keeping extraction temperature (40^0C), extractor bed geometry (B2) along with all other parameters of Table – 5.4 constant.



(P1=14.7MPa, P2=19.6MPa, P3=24.5MPa, Extraction Temperature = 40^0C, and ARSEF = 0.283)
Figure 7.3 Effect of extraction Pressure and operating time on Clove yield for Bed geometry B2

It shows that with increasing time of extraction, the yield of oil increases in all the cases. With increasing pressure slopes of the first parts of the overall extraction curves (OECs) increases and in case of the highest pressure level, the slope is very steeper. The increased slope of OEC with

increasing pressure is an indication of the faster rate of mass transfer or extraction of soluble oil molecules due to the increase in solubility of the oil in SCO_2 which increases the driving force of mass transfer. The yield of oil for a fixed period of extraction (210 min) for these three cases increases gradually as- 15.03% (in case of 14.7MPa operating pressure), 16.07% (in case of 19.6MPa operating pressure) to 16.83% (in case of 24.5MPa operating pressure).

❖ **Influence of Temperature**

In this work, the effect of extraction temperature on the extraction yield of clove buds was studied for three levels 35, 40, and 45^0C. Since extraction of clove oil is comparatively easier than many other essential oils, a lower range of temperature was considered in order to avoid thermal degradation of highly volatile heat-sensitive compounds. The perturbation plot (Fig. 7.2) suggests that the extraction temperature of SFE has an obvious positive impact on the clove oil yield within the chosen range. The nature of the curve illustrates that the yield of oil increases gradually with increasing temperature from 35^0C-45^0C, initially with an increasing order from 35^0C-40^0C and after that with a decreasing order above 40^0C. This may be due to the complexity in the characteristic of temperature resulting from its contradicting role on the rate of mass transfer to recover oil molecules. The primary counter effect of rising temperature is on the solubility of the solvent. Temperature rise tends to reduce the density of the solvent and thus reduce the solubility or driving force for mass transfer [Xu, 2011]. At the same time, vapour pressure of oil molecules increases with the increase of temperature, improving the solubility property of the solvent [Terada, 2010]. Thus the solvation power or solubility of supercritical solvents is the crucial property that controls the rate of extraction depending on the relative effects of density change and solute vapour pressure change upon change of temperature. At constant pressure, the solubility of the solute in supercritical solvent either increase or remain constant or decrease with the rise of temperature depending on which one is the predominant property between solute vapour pressure and density of the solvent [Xu, 2011]. Elevated temperature also induces an improvement in diffusive transport property of solvent molecules. Since viscosity-temperature are inversely related to each other with increasing temperature viscosity of solvent SCO_2 decreases [Heidaryan, 2011]. Increasing temperature thus improves the penetration of the solvent molecules through the plant matrix. As a result, the extraction of oil becomes easier. Hence it is concluded that selection of appropriate density and extraction temperature of solvent SCO_2 for solid-fluid extraction are the pivotal points controlling solvation power and selectivity; extraction time; colour, composition, and quantity of volatile oils [Cherchi, 2001; Wang, 2006].

The results of RSM study revealed that the positive impact of solute vapour pressure and enhanced diffusivity were able to defeat the negative impact of density drop and appreciable improvement in clove oil extract resulted with increasing temperature of extraction. Analysis of the responses of FC-CCD experiments showed that the average oil yields obtained at 35^0C, 40^0C, and 45^0C were 14.17%, 15.86%, and 16.05% respectively. It can be pointed out that the increase of the yield is more for the rise of temperature from 35^0C- 40^0C compared to rise from 40^0C-45^0C. Similar results can be observed from the experimental yields of clove oils obtained for annulus extractor bed with 0.75mm internal channel (B2) operated with an extraction pressure of 19.6MPa. The yields were 14.74%, 16.07% and 16.42% corresponding with the extraction

temperature of 35^0C, 40^0C, and 45^0C respectively. The rate of mass transfer was slow at 35^0C as this temperature is in the vicinity of the critical point of CO_2 (31.1^0C) where the effect of density, saturated vapour pressure, and diffusivity was less significant. With the rise of temperature from 35^0C to 40^0C rate of extraction increases rapidly. Hence it is obvious that in this range of temperature rise of saturated vapour pressure successfully beaten the adverse effect of density reduction, inducing an increase in solubility. The improved solubility speeded up the extraction process combining with diffusion coefficient, which also increased with increasing the temperature. Comparatively an inadequate extraction rate increment for the rise of temperature from 40^0C-45^0C indicated that the density drop due to temperature rise in this range started to compete closely with the rise of solute vapour pressure in this pressure range. However, the combined effect of diffusivity and solute vapour pressure as dominated over density drop resulting in the rise of yield with a reduced rate. Wenqiang (2007) also reported an increased yield of clove oil for temperature change 35^0C- 40^0C as temperature-solubility interferences are less significant at low temperature whereas negligible changes in oil yield in the range of temperature from 40^0C-50^0C.The maximum yield of clove oil obtained from different operating combinations was 18.05% at the highest operating temperature of 45^0C.

7.1.2.3 Effects of Two Factors Interaction on Clove Oil Yield

Multiple factors control the performance of a supercritical fluid extraction process. Effects of these factors are either independent or interactive. For a better understanding of the interactive effects between any two process factors and the response of the process, response surface plots are helpful. The surface plots are generally three-dimensional geometrical representation that provide a clear picture of the nature of dependency between the chosen input variables and corresponding response/s. These plots also have the potentials to point out the direction of enhancement of the process to meet the objective of optimization problems.

In the present study, three response surface plots were obtained which are useful to explain the effects of interactions of three combinations of input variables namely, temperature - pressure, temperature - extractor bed geometry and pressure - extractor bed geometry, on the performance of SFE to maximize the yield of clove oil.

- **Interaction effect of Extraction Temperature and Extractor Bed Geometry**

The three-dimensional response surface plot, Fig. 7.4, represents the interaction effect of two-factors, extraction temperature, and extractor bed geometry, on the yield of clove oil (%OY) within the range of their values selected for optimization. It is cleared from the graph that temperature and annulus extractor bed geometry both have appreciable influence on the clove oil yield. Increasing temperature from 35^0C to 45^0C for all the three types of extractor bed geometry used in the SFE module improves the yield of oil for a fixed extraction period. For a fixed operating temperature of the extractor, it is observed that as the value of ARSEF decreases or the ratio of radial to axial increases, the yield of oil obtained from clove buds increases.

The highest yield was obtained for the lowest value of ARSEF and highest extraction temperature. As stated earlier the lowest value of ARSEF (0.0238) indicates an annulus extractor bed arrangement with the largest internal channel (1.5mm) surrounded by 5.5mm diameter main

cylindrical extractor among the three different arrangements of the extractors and the highest value of ARSEF means the common type cylindrical extractor without any annular channel. The third type of extractor bed whose ARSEF value was intermediate of the above mentioned two types was also an annulus geometric arrangement to charge the feed to the extraction vessel. Hence it is clear from the results of FC-CCD experiments and surface plot, for a particular temperature to increase the yield introduction of annular geometry inside the extraction vessel was indeed a promising concept. For optimization of these two process parameters to maximize the yield, selection of higher temperature in the range 35^0C - 45^0C and annulus extractor bed geometry in place of conventional cylindrical geometry should be preferred.

Faster rate of mass transfer due to improvement in diffusion coefficient and driving force of oil concentration required for mass transfer at elevated temperature and reduction in diffusion path or mass transfer resistance and induced turbulence in the fluid bulk to favour convective transfer of solute due to introduction of annulus bed geometry might be the main reasons behind this result. Fig. 7.5 is a graphical representation of the OECs to compare the mass transfer rates of clove oil extraction for variation of two major operating parameters, extractor geometry and extraction temperature, at their two extreme levels [i.e., ARSEF values of 0.0238 and 0.0327; extraction temperature of 35^0C and 45^0C] maintaining all other operating conditions constant. These curves prominently showed that considerable faster rate of mass transfer with the highest yield was attained from the combined or interactive effect of lowest ARSEF value (B3) and highest extraction temperature (T3=45^0C). Interaction of lowest extraction temperature (35^0C) and highest ARSEF value (signifies the standard cylindrical geometry) reduces the rate of the mass transfer most here. Finally, it can be concluded that the incorporation of the annular geometry into the extractor of SFE module should not oppose the temperature effects on SFE from their normal trends, rather improve the efficiency of the extraction unit by increasing the yield for the same extraction time and same operating conditions.

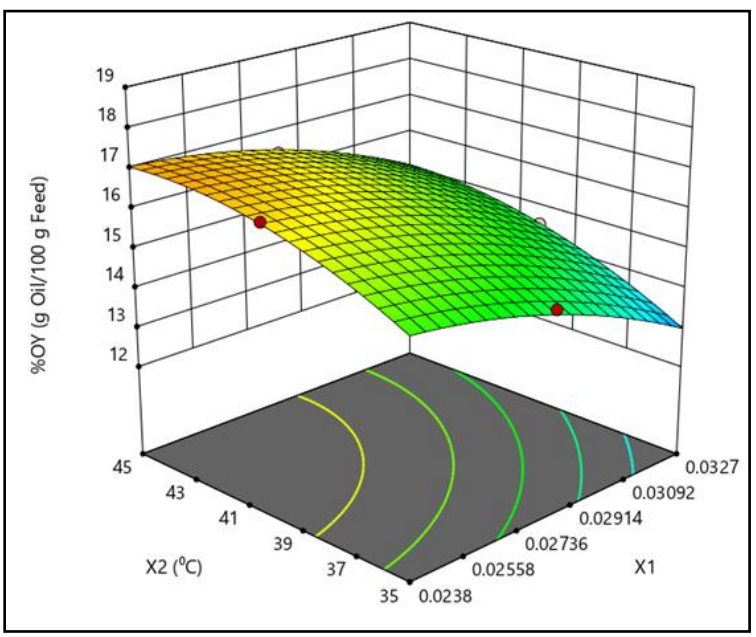
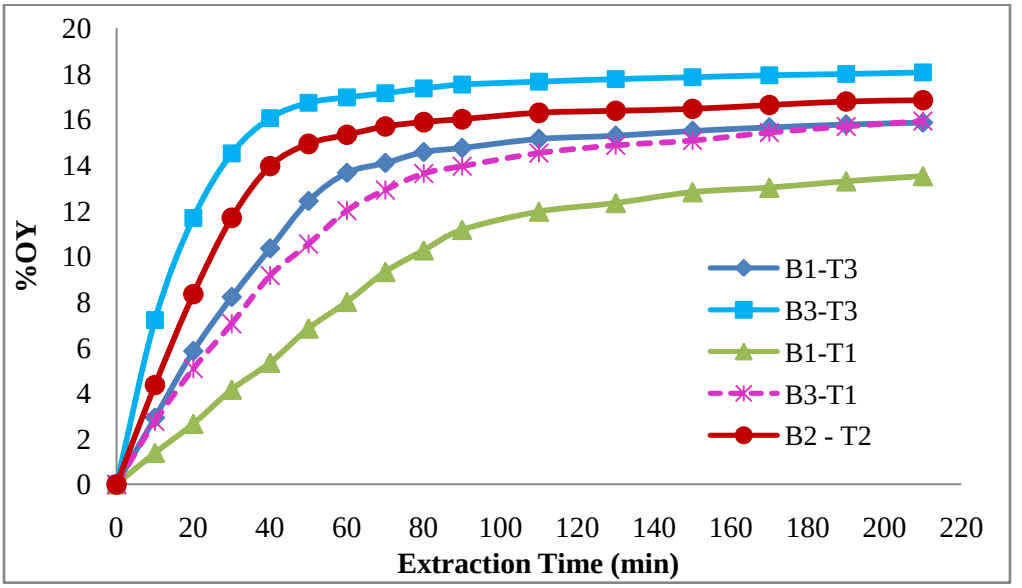


Fig. 7.4 : Temperature(X2) - Bed Geometry (X1) Interaction Effect on Extraction Yield



B1 (Cylindrical extraction vessel with ARSEF =0.0327), B2 (Annular extraction vessel with ARSEF =0.0283), B3 (Annular extraction vessel with ARSEF =0.0238), T1 = 35^0C, T2 = 40^0C and T3 = 45^0C

Fig. 7.5: Temperature-Bed Geometry Interaction Effect on Overall Extraction Curves of Clove oil

❖ **Interaction effect of Extraction Pressure and Extractor Bed Geometry**

Fig. 7.6 is a 3D response surface plot presenting the interaction effects of operational pressure of the extractor and geometry of extraction vessel simultaneously on the yield of clove oil obtained by extracting ground clove buds with the help of SCO_2. Extraction vessel is the principal part of the SFE module where the main extraction operation of valuable botanical fractions from the solid ground plant matrix is carried out applying optimized conditions of temperature, pressure, and solvent flow. The geometry of the extraction vessel plays a vital role in the performance of the extractor since geometry contributes on solid–solvent distribution, bed porosity, flow path, solvent residence time, mass transfer resistance, and axial dispersion. Since in this research work a typical pattern of extractor geometry that was distinct from the conventional form was introduced, studying the impact of extractor's geometry on usual behaviour of pressure on extraction is also of primary interest.

The surface plot indicates that interaction effect of increasing extraction pressure (within 14.7MPa – 24.5MPa) and advanced annulus bed geometry (i.e lower value of X1 or ARSEF) had more favourable approach to enhance the final amount of extract (%OY) compare to the interaction effect of increasing extraction pressure (within 14.7MPa – 24.5MPa) and traditional cylindrical bed geometry (i.e. largest value of X1 or ARSEF). It can be observed from the graph that with increasing extractor operating pressure from 14.7MPa to 24.5MPa the recovery of oil yields (%OY) was increased in all types of bed geometry. This trend of pressure–clove oil yield relation for cylindrical bed geometry (X1=0.0327 or B1) was mentioned in different works of

literature [Wenqiang, 2007; Yazdani, 2005]. For modified annulus extractor bed geometry (X1=0.0238 or B2 and X1=0.0283 or B3) the same trend between the pressure and yield of clove oil confirmed that pressure influence on the extraction mass transfer and yield would not break the general trend with the introduction of annulus geometry inside the extraction vessel (within these design values). For the same level of pressure from the three chosen values highest and lowest yields were always resulted from annulus extractor bed B3 (X1=ARSEF=0.0238) and cylindrical bed B1 (X1=ARSEF=0.0327), respectively. Lowest yield point on the graph indicated the lowest operating pressure level (14.7MPa) and cylindrical extractor B1 without any annular channel (i.e., X1=ARSEF=0.0327) whereas highest yield point on the graph indicated highest operating pressure level (24.5MPa) and extractor B3 which was developed with the annular channel of larger diameter (i.e., X1=ARSEF=0.0238). Thus it may be analysed that increasing nature of yield with the use of annulus bed geometry in place of the cylindrical type under increasing pressure condition has resulted since the reduction in diffusion path of molecules induced from annulus channel was coupled with the rise of solubility or driving force of mass transfer due to the rise of pressure.

Fig. 7.7 graphically shows how extractor geometry and extraction pressure influences each other and affects the mass transfer rates of clove oil extraction and hence the OECs.

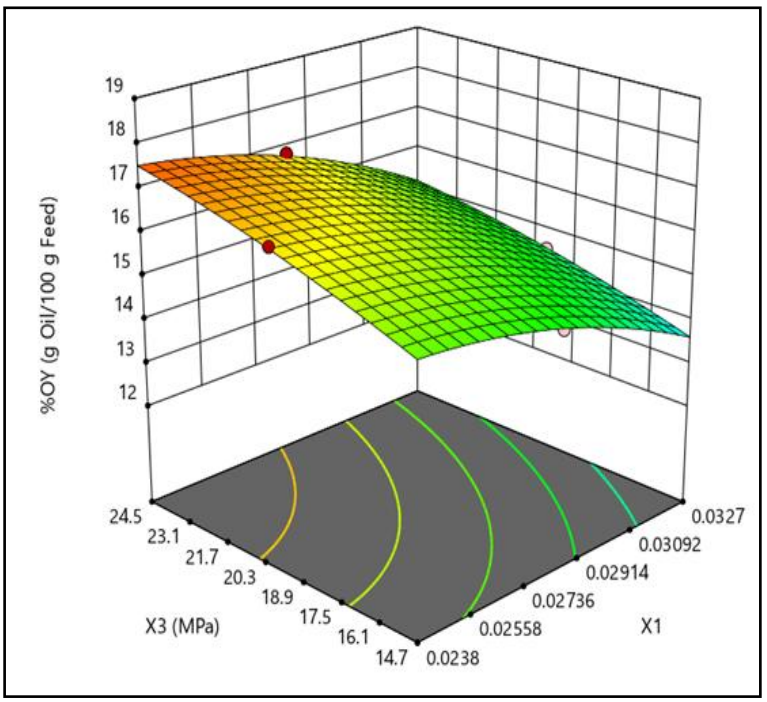
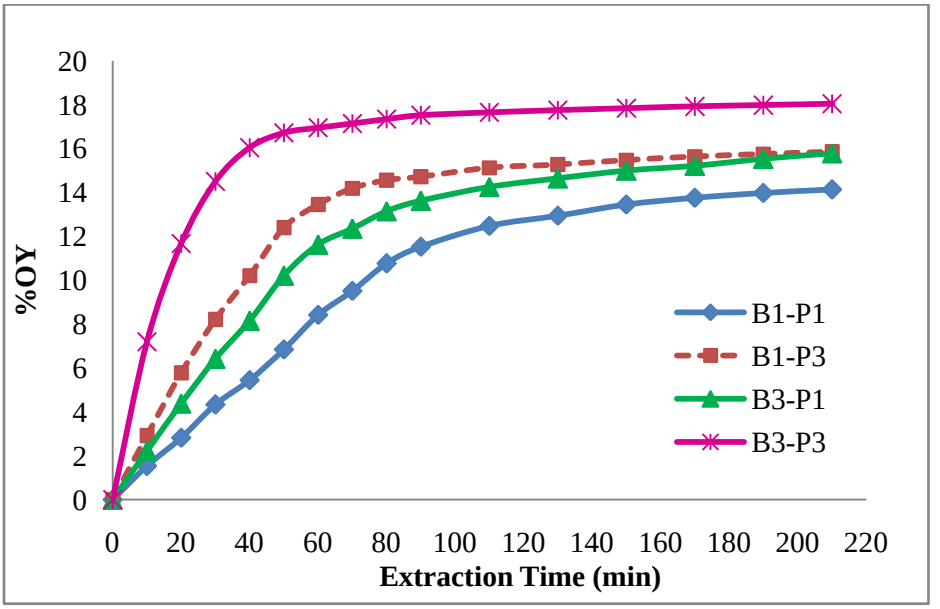


Fig. 7.6: Pressure(X3)- Bed Geometry (X1) Interaction Effect on Extraction Yield

The extractor geometry and extraction pressure effects on extraction process were presented here for their two extreme levels from FC-CCD database [i.e., ARSEF values of 0.0238 and 0.0327; extraction pressure of 14.7MPa and 24.5MPa] maintaining all other operating conditions constant. It can be observed that appreciable improvement in the rate of mass transfer and yield were achieved when highest pressure effect (P3=24.5MPa) was merged with the benefit of use of

annulus extractor geometry (B3 or lowest ARSEF value 0.0238). The initial steeper slope of OEC (B3-P3) obtained for annulus extractor bed B3 operated with the highest pressure of 24.5MPa clearly reflects the benefit of annulus geometry over cylindrical geometry (B1- P3) within the extraction vessel to accelerate the rate of mass transfer. Similar variation in OECs due to bed geometry alteration was observed from B1-P1 and B3-P1 plots obtained from low-pressure extraction.



B1 (Cylindrical extraction vessel with ARSEF =0.0327), B3 (Annular extraction vessel with ARSEF =0.0238), P1=14.7MPa and P3=24.5MPa

Fig. 7.7: Pressure - Bed Geometry Interaction Effect on Overall Extraction Curves of Clove oil

❖ **Interaction effect of Extraction Temperature and Pressure**

Fig.7.8 graphically presents the combined effects of the two most important mass transfer rate controlling parameters of SFE process, pressure, and temperature, on the yield of clove buds oil. Important observations came out from the graphical analysis are - (i) increasing temperature as well as pressure of extraction step within their design range of this investigation were effective to increase the amount of clove oil (%OY), (ii) keeping extraction temperature constant at any value of the selected level if pressure increases from 14.7MPa to 24.5MPa, extraction efficiency in terms of output yield increases, (iii) similar trend of oil recovery was also reflected for increasing operating temperature keeping extraction pressure constant, (iv)the highest yield of clove buds oil by SFE corresponds to the highest levels of temperature and pressure point (45^0C and 24.5MPa, respectively) in the graph, and (v) the lowest oil content was reflected at lowest point of the plot (35^0C and 14.7MPa, respectively).

These results can be explained from the knowledge of temperature-pressure individual as well as interactive effects on transport properties of both solutes and solvent molecules involved during

SFE. In the separation of soluble volatile components from plant matrix, temperature and pressure both affect the rate of mass transfer, and both contribute counter effects on the rate of transfer of molecules.

At constant temperature(below 45^0C) when the pressure of extractor was increased, the interaction between the bioactive solute molecules and solvent molecules of CO_2 increased due to the decrease of molecular distance inside the compressed vessel that leads to an increase in density as well as solvation power of CO_2 [Liza, 2010]. Increasing solubility of the solute molecules means increasing equilibrium concentration of the solute in the solvent, favouring the rate of mass transfer by improving the driving force of concentration difference. Dense medium under high pressure also initiated an adverse effect on the rate of mass transfer by reducing the diffusion coefficient. At high pressure (above 30MPa), the increase of molecular collision frequency leads to the reduction in the mean free path of molecular transport, resulting in a slower rate of diffusion or less efficiency in SFE [Rezaei, 2000]. Elevated pressure (above 30MPa) is also responsible for reducing the extraction efficiency by increasing the solubility of various solutes other than targeted analytes. As a result, co-extraction of high molecular weight components produced a complex extract and presence of co-extracted molecules unfavourably changed the solubility value of the molecules of interest [Hamburger, 2004; Pourmortazavi, 2007; Zhang, S., 2010]. At high pressure levels sometimes solute and highly compressed CO_2 molecules interaction became repulsive, inducing complexity in the extraction process [Xu, 2011]. High pressure increased the viscosity of SCO_2 that interrupt the easy penetration of the solvent molecules inside the solid matrix [Heidaryan, 2011].

For controlling the rate of mass transfer of SFE, temperature effects on density, solubility, vapour pressure, diffusivity, and viscosity need to be considered. At constant pressure, the increased temperature reduces the density of the solvent and simultaneously increases the saturation vapour pressure of solute molecules in SF. Reduced density opposed the favour of vapour pressure in improving the solubility of solutes of interest and hence the rate of mass transfer. Thus for temperature rise at constant pressure, the effect of solubility on the rate of mass transfer may or may not be favourable, or may remain unaltered depending on whether the vapour pressure of solute or the CO_2 density is predominant factor [Bimkr, 2012, Pourmortazavi, 2007; Terada, 2010; Thana, 2008; Xu, 2011; Zang, 2010]. Increasing temperature also influences the diffusion of solvent molecules in the solid matrix, which improves the extraction process [Leo, L., 2005]. The viscosity of SCO_2 molecules decreases with increasing temperature at constant pressure [Heidaryan, 2011] which results in an increase in diffusion coefficient and helps the mobile phase of SCO_2 to penetrate easily through the solid matrix and recover the solutes.

From this discussion, it can be pointed out that the rise of both temperature and pressure of extraction has some positive as well as negative impact on extraction. Depending on the nature of solute molecules, the yield of extract of SFE from plant material significantly increases -(i) with increasing pressure at a given temperature where positive impact of increased pressure on solubility is predominant over the negative impacts of elevated temperature and elevated pressure, (ii) with increasing temperature at a given pressure where increased solubility due to sufficient vapour pressure overcomes the negative impact of reduced density on it. At the lower level of pressure, oil yield may decrease with increasing temperature due to the strong influence

of density reduction of SCO_2 on solubility that is not even balanced by the positive impact of the solute vapour pressure. At higher level of pressure (commonly above 30MPa) with increasing temperature after a particular value (commonly about 45^0C), the yield of oil starts to fall due to the counter-effect of high temperature on solubility and that of high pressure on various factors such as diffusivity, viscosity, solute-solvent repulsive effect, that lead to the reduction of extraction efficiency. For these reasons, to maximize the yield of oil from plant material, the optimum condition of pressure and temperature need to be evaluated.

In the present study, throughout the region of pressure (highest value 24.5MPa) and temperature (highest value 45^0C) selected for extraction, the effective density of SCO_2 and vapour pressure of oil molecules jointly induced the increase of solubility. As a result, the oil yield increased with the increase of both pressure and temperature, and the highest yield was obtained at the highest levels of extraction temperature and pressure.

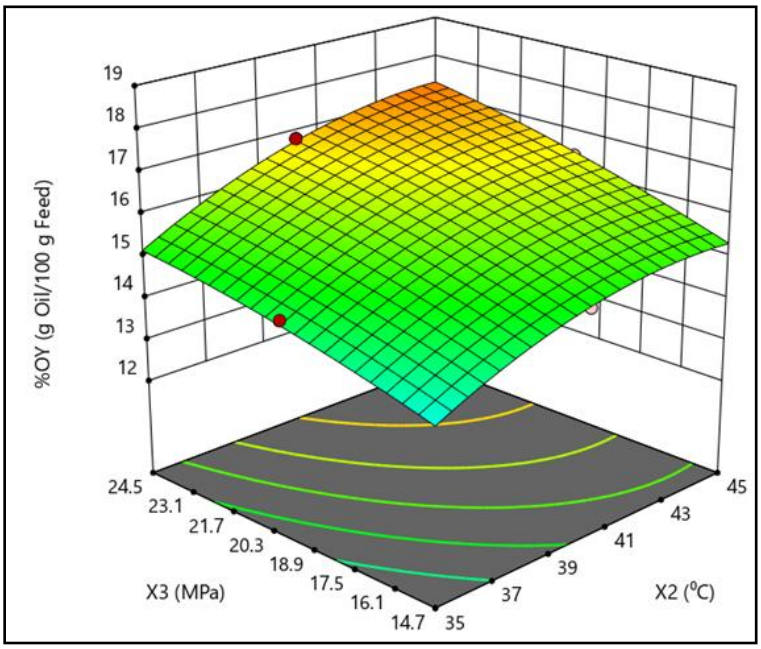


Fig. 7.8: Temperature (X2)- Pressure (X1) Interaction Effect on Extraction Yield

Fig.7.9 - 7.11 show the interaction effects of temperature – pressure on the rate of mass transfer with the help of OECs for two different type annulus bed geometry and conventional cylindrical bed geometry, B2, B3, and B1, respectively. For all the plots it was observed that all OECs had some similarity in their nature, the main difference in these curves was developed from the rate of mass transfer under varying extraction condition of pressure and temperature. These differences were graphically reflected in the slopes of initial steeper parts of the curves. Steeper the slope of OEC, faster is the rate of extraction and higher is the resulted yield of oil for constant extraction time.

From Fig.7.9, it is observed that highest pressure (P3=24.5MPa) or highest temperature (T3=45^0C) of extraction when combined with intermediate temperature (T2=40^0C) and pressure

(P2=19.6MPa), respectively, yielded almost the same extract due to the same rate of mass transfer and thus, resulted in similar type OECs (T3-P2, P3-T2). A similar trend could also be noticed for the lowest temperature (T1=35^{0}C) and lowest pressure (P1=14.7MPa) extraction curves (T2-P1, T1-P2). With increasing pressure from P1 to P3at constant temperature (T2), the rate of mass transfer and hence the yield of oil increased gradually in the order 15.03% for lowest pressure (T2-P1), 16.07% for intermediate pressure (T2-P2), and 16.83% for highest pressure (T2-P3). A similar trend was also reflected with increasing temperature at constant pressure from the OECs, T1-P2, T2-P2, and T3-P2, respectively.

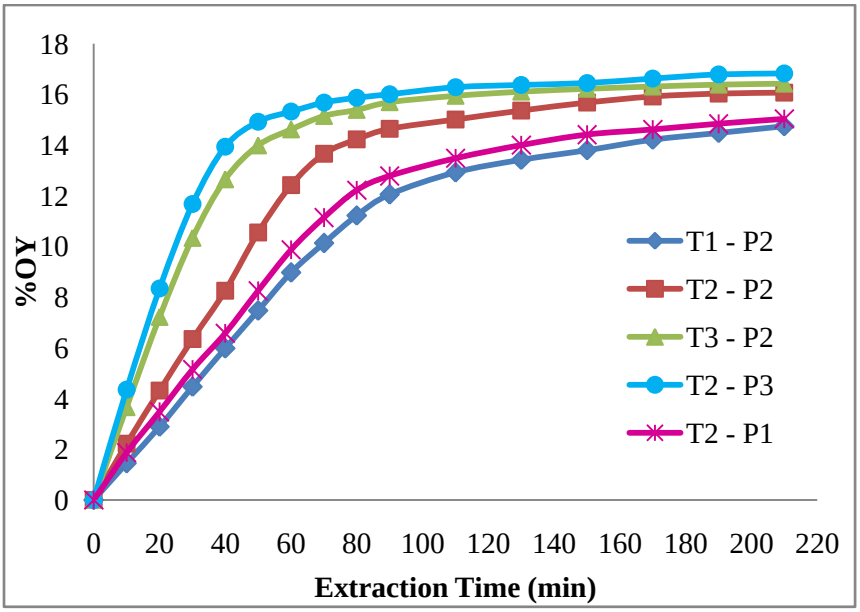


Fig.7.9: OECs of Clove oil obtained from Annulus Extractor Bed B2

Fig.7.10 shows that the fastest and slowest rates of mass transfer and corresponding maximum (18.05%) and minimum (14.52) yields of clove oil resulted from combined highest levels of temperature –pressure (T3-P3) and lowest levels of same factors (T1-P1), respectively. It was observed that highest pressure or the highest temperature of extraction when combined with lowest levels of extraction temperature and pressure, respectively, yielded almost same amount of clove oil due to the same rate of mass transfer and the resulted OECs (T1-P3, T3-P1) were almost similar with each other. It was also noticed from the graph that the rate of mass transfer and yield of oil for intermediate temperature –pressure condition (T2-P2) were notably higher compared with the extraction operating combinations as per FC-CCD where anyone parameter between temperature and pressure was chosen in the lowest level (i.e., T1-P3 or T3-P1).

Fig.7.11 is a graphical representation of OECs generated from the extraction experiments which were conducted with the normal cylindrical feed bed under varying combinations of operating temperature and pressure following FC-CCD. The operation conditions were similar like that of annular bed (B3). The graphs indicate that the fastest and slowest rates of mass transfer and corresponding maximum (15.85%) and minimum (12.11) yields of clove oil were attained from combined highest levels of temperature – pressure (T3-P3) and lowest levels of same factors (T1-P1), respectively. It was also observed that the rate of mass transfer and yield of oil for

intermediate temperature –pressure condition (T2-P2) are higher compared with the extraction operating conditions at least one factor between temperature and pressure was chosen in the lowest level (i.e., T1-P3 or T3-P1 or T1-P1). If the resulted yields of extraction for two bed geometries, B1 and B3, are compared from the graphs, quite a large oil yield could be noticed for all runs in case of annulus extraction vessel B3 than the same for cylindrical extraction vessel B1. This difference in the extracted quantity is mainly due to the differences in the rate of mass transfer between these two bed geometries.

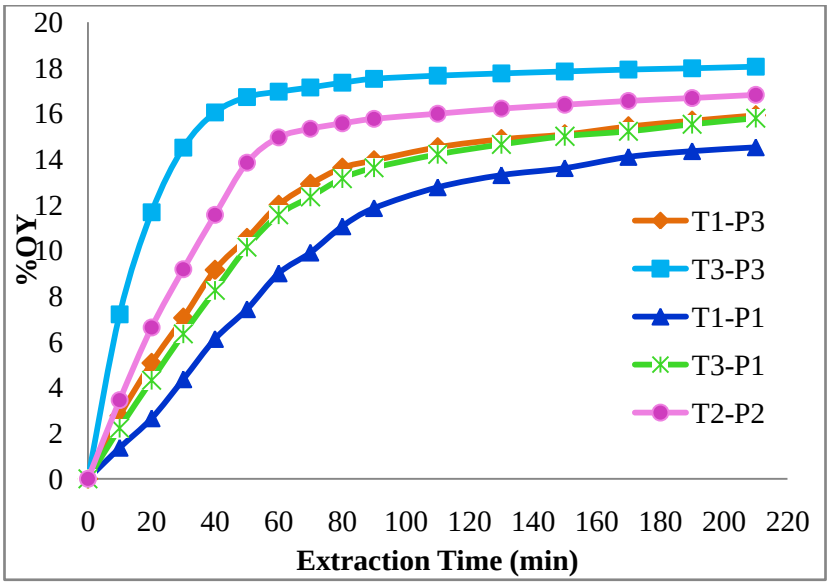


Fig. 7.10: OECs of Clove oil obtained from Annulus Extractor Bed B3

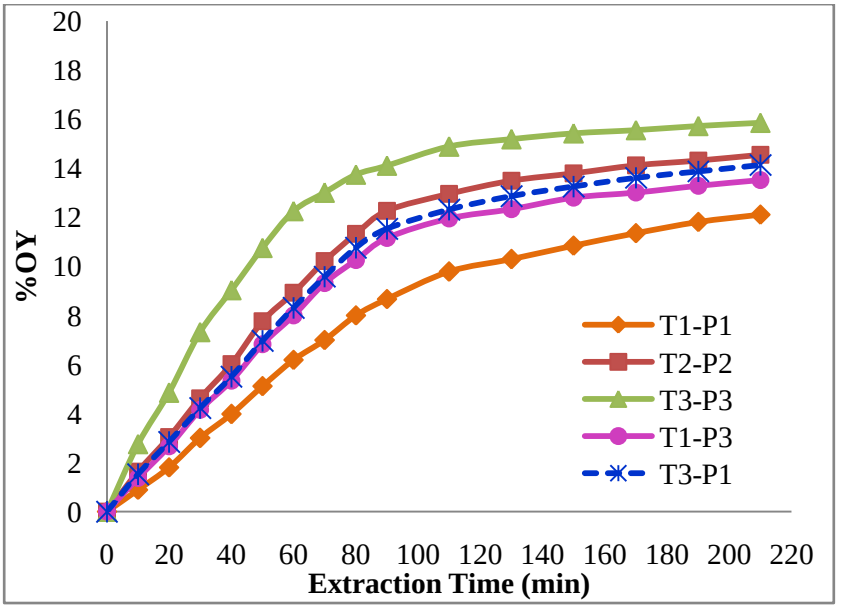


Fig. 7.11: OECs of Clove oil obtained from Conventional Cylindrical Extractor Bed B1

7.1.2.4 Optimum Conditions of Operating Parameters to Maximize the Yield

Based on the above study, Design-Experts numerical optimization of the input factors (X1=ARSEF which is related with the geometry of the extractor, X2=extraction temperature, and X3=extraction pressure) was carried out to anticipate the optimal condition of the process in order to obtain the maximum yield of clove oils. Derringer and Suich developed the optimization method used in this software, described by Myers, Montgomery, and Anderson-Cook in Response Surface Methodology, 3rd edition, John Wiley and Sons, New York, 2009.The optimum conditions of input factors identified were a ARSEF value of 0.0238 that corresponds with the annulus extraction vessel (B3), an extraction temperature of 44.72°C and an extraction pressure of 24.5MPa.The maximum predicted extraction yield of clove oil was 17.981%. Extraction of clove buds under these optimal conditions, which were close to the highest operating levels of temperature and pressure using annulus extractor bed geometry (B3) resulted a yield of 18.05%. The results are provided in Table -7.6.

Table-7.6: Optimum points of operating parameters to maximize the response (%OY)

%OY	ARSEF	Pressure (MPa)	Temperature (°C)
17.981	B3 (0.0238)	24.5	44.72

7.1.3 Effect of Bed Geometry on Supercritical Fluid Extraction Kinetics

The yields of clove oil obtained at different intervals of time of extraction runs conducted to study the effect of bed geometry on SFE kinetics were provided in Table-7.7. All the kinetic experiments were performed to predict the rate of extraction of clove oil for three different geometric configurations B1, B2, and B3 as described in this study. Experiments were designed to determine the effect of the bed geometry on the OECs for three different extraction temperatures (T1=35°C, T2=40°C, and T3=45°C). Fig.7.12 shows the influence of bed geometry on the yield of oil extracted at various levels of temperatures. It was noticed from the graphs that for all values of temperature, the resulted yields of oils were more in case of both types of extractor beds with annulus geometry (B2 and B3) than the traditional cylindrical bed geometry (B1).

The relation between operating time and the corresponding yield of oils for three different extraction temperatures were plotted as OECs (Fig.7.13, Fig.7.14, and Fig.7.15). A significant influence of extractor fixed bed geometry on SFE kinetics was noticeable from the plots. The OECs were fitted with two straight lines using the graphical method. The first line passing through the origin was mentioned as the constant extraction rate (CER) period, where the main mass transfer occurs at the surface of the molecules and mechanism is convection in the fluid phase. The second portion of the curve was termed as falling extraction rate (FER) period, where the mass transfer resistance increases due to the depletion of oil layer from the surface of the solid matrix and the extraction occur mainly by diffusion inside the solid particle. [Farias-Campomanes, A.M., 2015; Zabot, G.L.,2014]

In OEC, the information about the CER period is very much essential because it represents the period of extraction with highest and constant rate and greater productivity. Thus, CER period contributes remarkably to the manufacturing cost of SFE process. The economic feasibility study of some commercial SFE processes considered that continuation of the extraction process to exhaustion is not always cost-effective. In many commercial SFE processes, it was considered that the extraction of plant material could be continued up to recovery of 90% of the extractable solute part. The increased operational costs involved with the prolongation of extraction to recover the rest portion of the extract could not always compensated by the improvement in the yield quantity [Valle, 2005]. The most significant amount of extract in unit time is possible to recover in this CER period because solute molecules are dissolved at a faster rate as they are easily accessible by the solvent. The resulting extraction yields of kinetic experiments and calculated parameters of kinetic study for the CER period in terms of t_{CER}, R_{CER}, and Y_{CER} are furnished in Table -7.8. The duration of CER period (t_{CER}) was determined by the intersection of two lines.

For all the three levels of temperature (T1, T2, and T3), OECs differed greatly among the beds B1 (representing conventional cylindrical bed geometry), B2 and B3 (representing annular extractor bed geometry of different configurations). The main reason behind this deviation in OECs might be the difference in bed geometry. Comparative studies on the OECs of three beds reveal that t_{CER} is lowest in case of annulus extraction vessel B3 and highest in case of cylindrical extractor bed B1 for all levels of temperature studied. For extraction temperature of 45°C, t_{CER} was only 41 minutes for annulus extraction vessel B3 compared to 57.5 minutes for cylindrical extractor bed B1. On the other hand, the rate of mass transfer (R_{CER}) and percentage recovery of oil w.r.t. the global yield ($\%Y_{CER}$) were highest in case of bed geometry B3 and lowest in the case of cylindrical bed geometry B1 for the same level of temperature studied. This happened due to the reduction in mass transfer resistance within the annulus extraction path as compared with cylindrical extractor bed, inducing faster recovery of oils in annulus extractor bed. In the CER period, $\%Y_{CER}$ value corresponding with the extraction temperature T3 was sufficiently high about 83.68% using annulus geometry as compared to 70% recovery of oil applying the same operating conditions and cylindrical geometry.

Another important observation identified from the calculated kinetic parameters indicates that with increasing temperature for all types of bed geometry, t_{CER} gradually decreases and other kinetic parameters such as R_{CER}, Y_{CER} and total yield (%OY) extracted in the constant period of 210 minutes, increase. The positive effect of temperature (up to 45^0 C) on the extraction yield of clove oil was reported in the research article of Wenqiang (2007). Grosso (2008) also reported that increasing extraction temperature helps to promote the faster release of the monoterpene hydrocarbons (the main component of clove oil eugenol in this case) from the botanical materials. It validates the increased values of R_{CER}, Y_{CER} with increasing temperature during CER period.

As there is no depletion of solute molecules in the constant extraction rate period and surface diffusion controls the rate, the manifestation is quite prominent in CER rather than FER where pore diffusion controls the rate. From the rate curves, it was also noted that though there are increasing trends of R_{CER} and Y_{CER} from B1 to B3, it is not linear but of asymptotic in nature. This is quite obvious as larger voids can invite ill effects of channelling.

Table-7.7: Extraction Time vs. Yields of Clove Oil Data obtained from SFE Kinetic Studies

Time (min)	Temperature, T1			Temperature, T2			Temperature, T3		
	B1	B2	B3	B1	B2	B3	B1	B2	B3
	% OY	% OY	% OY	% OY	% OY	% OY	% OY	% OY	% OY
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	1.12	1.45	1.95	1.5	2.15	3.45	2.61	3.66	4.56
20	2.39	2.89	3.82	2.89	4.09	6.63	5.03	7.22	9.06
30	3.63	4.47	5.71	4.26	6.12	9.18	7.42	10.34	12.75
40	4.78	5.98	7.54	5.74	8.12	11.56	9.54	12.65	14.72
50	6.05	7.47	9.27	7.24	10.11	13.85	11.25	13.98	15.45
60	7.23	8.97	10.61	8.62	12.09	14.95	12.64	14.62	15.86
70	8.38	10.13	11.65	10.01	13.65	15.33	13.17	15.15	16.23
80	9.43	11.22	12.57	11.17	14.23	15.56	13.61	15.38	16.53
90	10.28	12.09	13.30	12.26	14.64	15.76	13.92	15.69	16.78
110	11.21	12.88	13.81	12.95	15.01	15.99	14.21	15.94	16.89
130	11.65	13.42	14.23	13.49	15.36	16.21	14.46	16.1	16.99
150	12.08	13.85	14.54	13.79	15.67	16.38	14.67	16.22	17.08
170	12.45	14.21	14.78	14.12	15.92	16.55	14.81	16.31	17.18
190	12.78	14.52	15.10	14.31	16.03	16.68	14.93	16.38	17.22
210	13.1	14.74	15.33	14.51	16.07	16.81	15.01	16.42	17.26

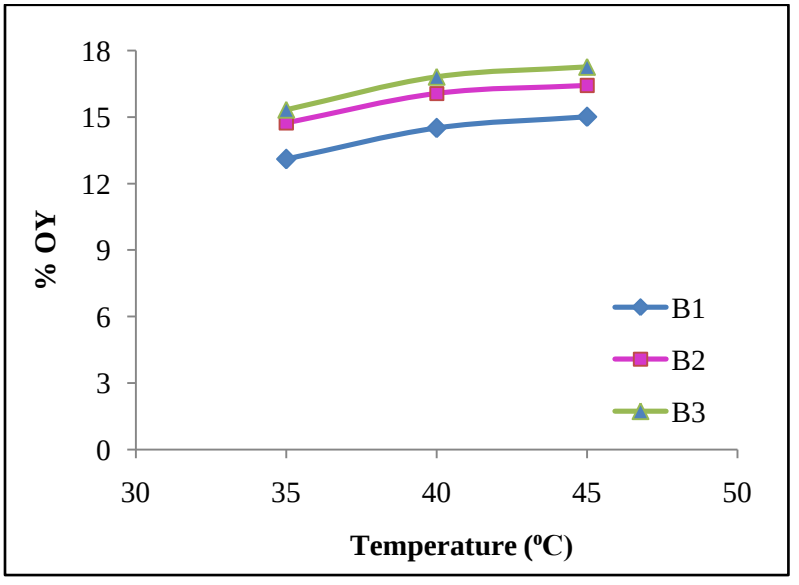


Fig.7.12: Effect of Extractor Bed Geometry on the Clove Yield obtained in Kinetic Studies

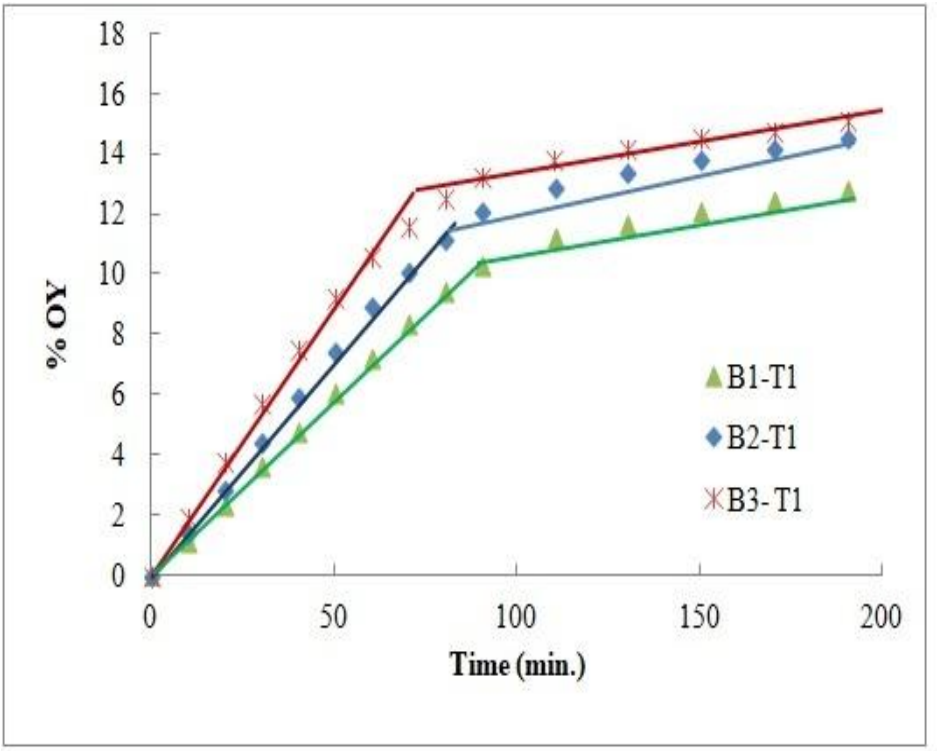


Fig. 7.13: Effect of Extractor Bed Geometry on the OECs obtained from Kinetic Studies at 35^0C

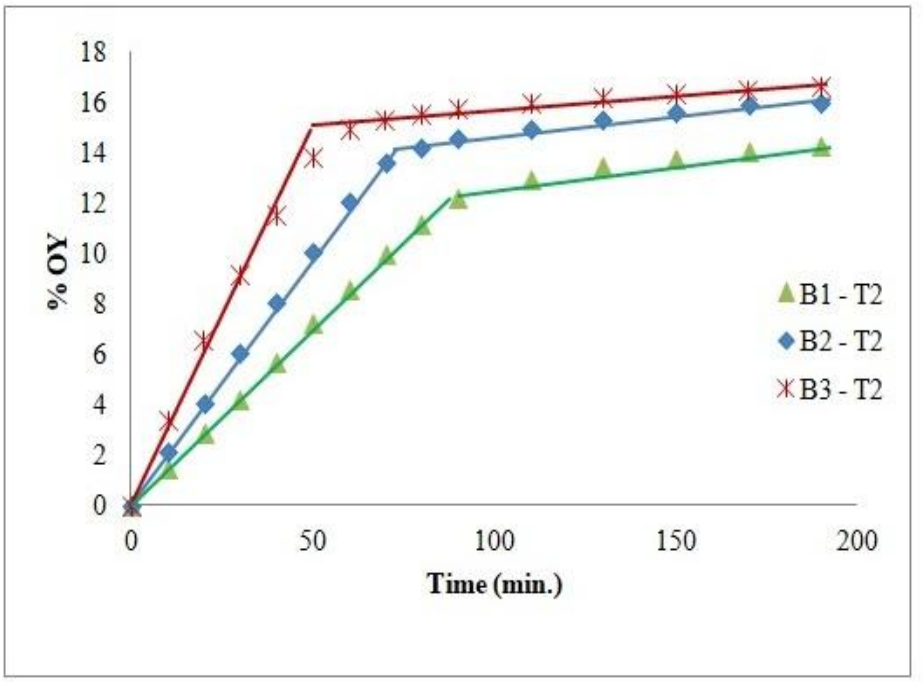


Fig 7.14: Effect of Extractor Bed Geometry on the OECs obtained from Kinetic Studies at 40^0C

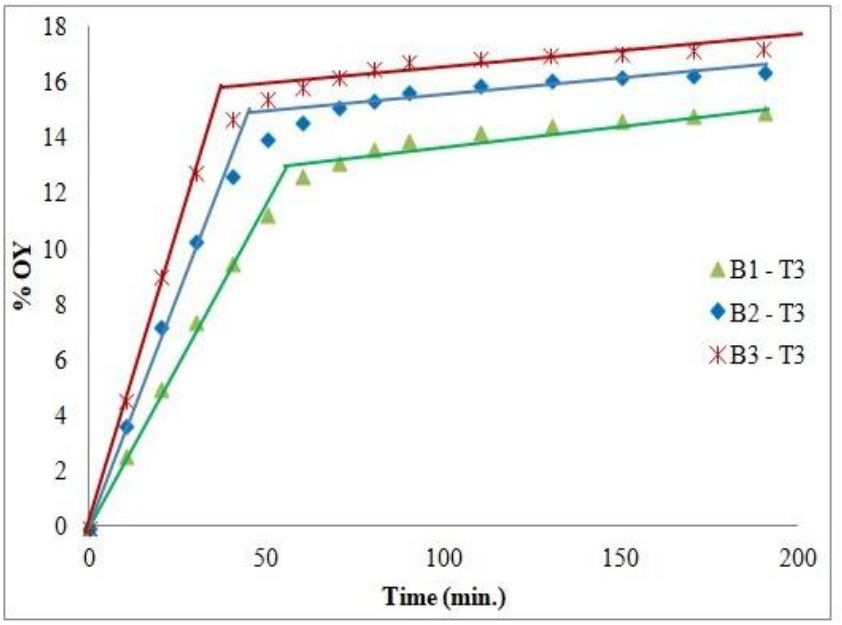


Fig.7.15: Effect of Extractor Bed Geometry on the OECs obtained from Kinetic Studies at 45^0C

Table-7.8: Experimental and Estimated data of SFE Kinetic Studies

Run	t_{CER} (min)	R_{CER} (10^{-6} kg/s)	Y_{CER} (gm Oil obtained during CER / 100gm Feed)	$\%Y_{CER}$ $\left[\frac{(Y_{CER}\times 100)}{Global\ Yield}\right]$	% OY (gm Oil/ 100gm Feed)
B1-T1	95.0	11.58	11.0	57.89	13.10
B2-T1	85.0	14.47	12.3	64.74	14.74
B3-T1	73.0	17.67	12.9	67.89	15.33
B1-T2	87.5	14.29	12.5	65.79	14.51
B2-T2	72.5	19.59	14.2	74.74	16.07
B3-T2	52.0	29.04	15.1	79.47	16.81
B1-T3	57.5	23.13	13.3	70.00	15.01
B2-T3	47.5	30.95	14.7	77.37	16.42
B3-T3	41.0	38.78	15.9	83.68	17.26

Here CER, Constant extraction rate; $t_{CER,}$ constant extraction rate period; R_{CER}, the rate of extraction during CER; Y_{CER}, % yield achieved during CER; $\%Y_{CER}$, % yield recovered w.r.t. global yield during CER; and %OY, percentage of extracted yield.

B1-T1 : SCO_2E using extractor feed bed B1at extraction temperature T1 (35^0C);

B2- T1 : SCO_2E using extractor feed bed B2at extraction temperature T1 (35^0C);

B3- T1 : SCO_2E using extractor feed bed B3at extraction temperature T1 (35^0C);

B1-T2 : SCO_2E using extractor feed bed B1at extraction temperature T2 (40^0C);

B2-T2 : SCO_2E using extractor feed bed B2at extraction temperature T2 (40^0C);

B3-T2 : SCO_2E using extractor feed bed B3at extraction temperature T2 (40^0C);

B1-T3 : SCO_2E using extractor feed bed B1at extraction temperature T3 (45^0C);

B2-T3 : SCO_2E using extractor feed bed B2at extraction temperature T3 (45^0C).

B3-T3 : SCO_2E using extractor feed bed B3at extraction temperature T3 (45^0C).

7.1.4 Effect of Particle Size of Clove Buds on Supercritical Fluid Extraction

Preliminary tests were performed to determine the performance of modified annulus extractor bed geometry as compared to conventional cylindrical geometry and find out suitable operating conditions (temperature and pressure) to maximize the yield of oil. The particle size of the raw plant material is another important factor that contributes a vital role on increasing the yield of extraction. Sometimes its influence is very much crucial when compared with other factors like pressure, temperature and solvent flow rate of extraction [Priyanka, 2018]. Present study revealed the effect of particle size under the modified geometric configuration of the bed. The experimental results obtained from SFE runs conducted with three different particle sizes of clove buds and annulus bed geometry (B3) were provided in the Table -7.9.

Table-7.9: Effect of Particle Size on Yield of Clove Oil

Extraction Time (Min)	DP1 = 0.64 mm	DP2 =1.0 mm	DP3 = 1.5 mm
	% OY	% OY	% OY
0	0.00	0.00	0.00
10	4.56	3.45	2.47
20	9.06	6.52	4.57
30	12.75	8.92	6.42
40	14.72	10.18	7.80
50	15.45	11.43	9.10
60	15.86	12.28	9.82
70	16.23	12.60	10.57
80	16.53	13.07	10.94
90	16.78	13.37	11.20
110	16.89	13.92	11.72
130	16.99	14.20	12.05
150	17.08	14.67	12.34
170	17.18	14.92	12.58
190	17.22	15.12	12.71
210	17.26	15.24	12.82

The yields as a function of particle size were plotted in Fig.7.16 that reflects the effect of particle size on the total yield (%OY). As expected, it was observed that the particle size and extraction yield were inversely correlated with each other. As a result, the extraction yield of clove oil increased with decrease in particle size (Fig. 7.16).

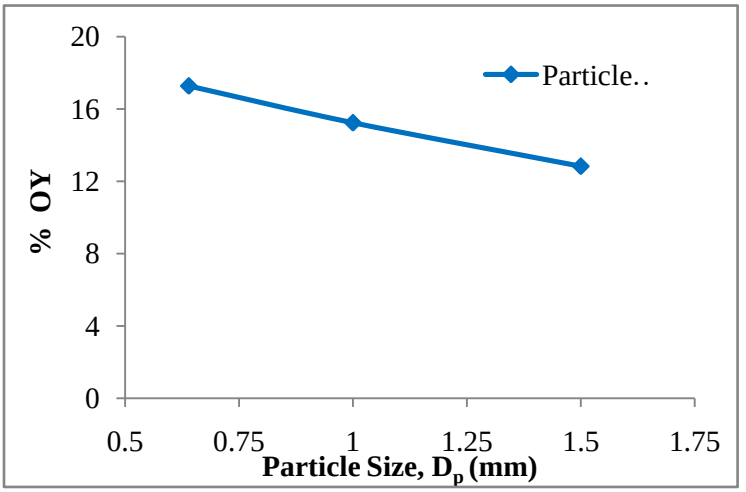


Fig.7.16: Effect of Particle Size on Yield of Clove Oil Extracted using SCO_2

To generate the rate curves or OECs, the yields of clove oil obtained from different particle sizes were plotted as a function of extraction time for each particle size separately in Fig.7.17. At the beginning of the extraction, deviations in yields for different particle sizes were comparatively less. It signifies that the solvent SCO_2 was almost saturated with clove buds oil before leaving the extraction vessel. With increasing extraction time, the yield of clove oil increased for all the particle sizes, but the rate of extraction gradually dropped with increasing the size of ground clove particles.

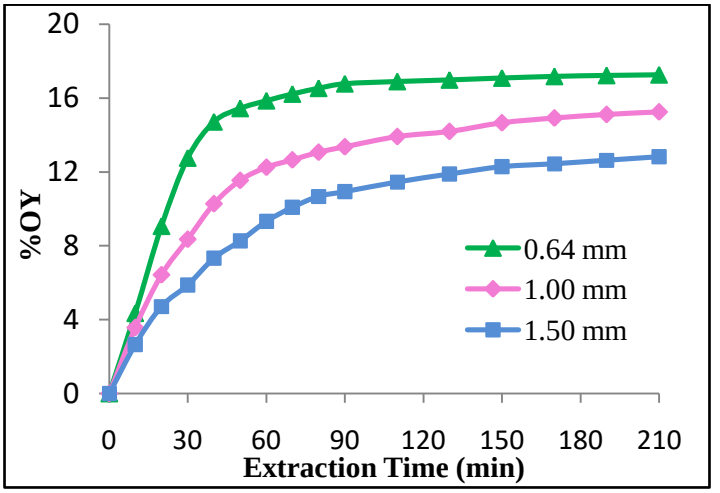


Fig.7.17: Effect of Particle Size and Extraction Time on Yield of Clove Oil obtained from Extractor B3

The maximum yield of clove oil obtained from these runs was 17.26% for the particle size of 0.64 mm compared to 15.24 % for 1 mm and 12.82 % for 1.5 mm particle sizes under the same operating conditions. The increasing yield of oil with decreasing particle size resulted from the benefit of milling the whole clove buds. The milling of plant material before extraction induced two favourable conditions to improve the extraction rate – (i) it increases the interfacial area of contact between solid and solvent phase and (ii) it helps to rupture the cell walls of whole buds. Thus more oil molecules are exposed to the surface from the interior of the cell, become accessible to the solvent and easily extracted, increasing the extraction yield [Reverchon, 1997; Priyanka, 2018]. The strong dependence of the rate of mass transfer on the size of particles appears to be more prominent when internal diffusion constitutes the controlling step of the extraction process [Kandiah, 1990]. The milling operation of the plant matrix reduces the diffusion path of the internal mass transfer. Contrary, when external mass transfer controls the SFE process, the rate of extraction is less affected by the particle size [Reverchon, 1997]. For the larger sized particles, the available surface area for mass as well as heat transfer is small, and the solvent CO_2 experiences a higher resistance when diffused through the unbroken cells to extract the oil molecules present in the inner part of the clove buds. Hence the process of extraction resulted with a lower yield of oil [Del Valle, 2002].

Analysis of the OECs indicates that in the initial period where the slope was steep, the recovery of clove oil is faster. In this period oil molecules which are mostly present on the surface of the ruptured cells were extracted under the control of surface diffusion, and convection in the solvent phase and no internal diffusion occurs as long as the surface of the solid matrix remains saturated with the oil molecules. The second part of the curves presents the slow extraction phase due to depletion of the oil layer from the surface to the interior layer of the matrix at a faster rate and internal diffusion controlling the extraction rate. Thus milling operation reduces the time of extraction and improves the yield of oil by strongly influencing the rate of extraction from broken cells of clove buds.

The results of the present study recommend the particles of milled clove buds of 0.64 mm average diameter as desired particle size from the chosen categories to maximize the recovery of yield.

7.1.5 Chemical Composition of Clove Buds Oil

The yield of oil obtained using the supercritical solvent $CO_2$is generally lower than that of Soxhlet extraction. Optimization of the SFE process parameters may improve the quantity of extract close to that of Soxhlet extraction. In this study, the highest yield of 18.05% clove oil was obtained at 45^0C temperature and 24.5MPa pressure using annulus extraction vessel B3 as compared to 19% extract obtained from solvent extraction using Soxhlet Extractor. Applying same operational conditions conventional cylindrical extraction vessel yielded a maximum of 15.85% clove oil. Thus applying modified bed geometry, it is possible to extract 95% of the recoverable oil, which is almost 12% larger in quantity than the conventional cylindrical extractor. The higher recovery of yield using supercritical fluid resulted from – (i) the unique solvation property of SCO_2 that is similar to that of the liquid and can be tuned easily by changing the pressure and temperature slightly to induce selective separation of the targeted bioactive

components in concentrated form, (ii) higher diffusivity that contributed to faster rate of mass transfer and (iii) negligible viscosity that enable the supercritical fluid to penetrate easily into the depth of the solid ground feed sample.

SCO_2E of clove (Syzygium aromaticum) buds had been carried out previously by some authors as –(i) it has wide range of applications in food, cosmetic, and pharmaceutical industries and (ii) selection of clove buds as "model raw material" is ideal for SCO_2E studies for its high oil content and ease extraction from its matrix. These studies evaluated the effect of different operating conditions on the yield of oil using SCO_2 solvent and its major bioactive components [Della, 1998;Gopalakrishnan, 1990; Huston, 1991; Li, 2015; Martinez, 2007;Moyler, 1986; Prado, 2011; Reverchon & Marrone, 1997; Wenqiang, 2007; Yazdani, 2005; Zabot, 2014]. Extraction temperature, pressure, and time of extraction were varied mostly in the range from 35-50^{0}C, 10–50MPa and 30 minutes-20 hrs (for an exhaustive run), respectively. Yazdani studied high temperature (52-141^{0}C) effect on clove extract and its constituents at 19MPa. The yields of clove oil and its main component eugenol obtained in different studies were varied from 11.2-24% and 53.8-72.74%, respectively. High temperature study by Yazdani yielded a clove oil at an optimum temperature of 80^{0}C that contains only two compounds (eugenol, 86.7% and eugenyl acetate, 13.3%).

The clove oil contents obtained from SCO_2E in this study were varied from 12.11% -18.05% for a constant extraction period of 210 minutes under varying pressure and temperature of extraction and bed geometry of the extractor. The highest yield obtained in this study was as high as 95% of the total oil content evaluated by Soxhlet extraction using annulus geometry inside the extraction vessel at a temperature of 45^{0}C and pressure of 24.5MPa. Thus the results of clove oil yields obtained through SFE were confirmed to the reported results.

Fig.7.18 is provided as noticeable changes of oil-rich clove buds were observed before and after carrying out the supercritical fluid extraction experiments. The dark colour of oil-rich clove buds was faded significantly after recovery of oil with the help of solvent SCO_2. The photograph of sample clove oil obtained from supercritical carbon dioxide extraction is given in Fig.7.19.

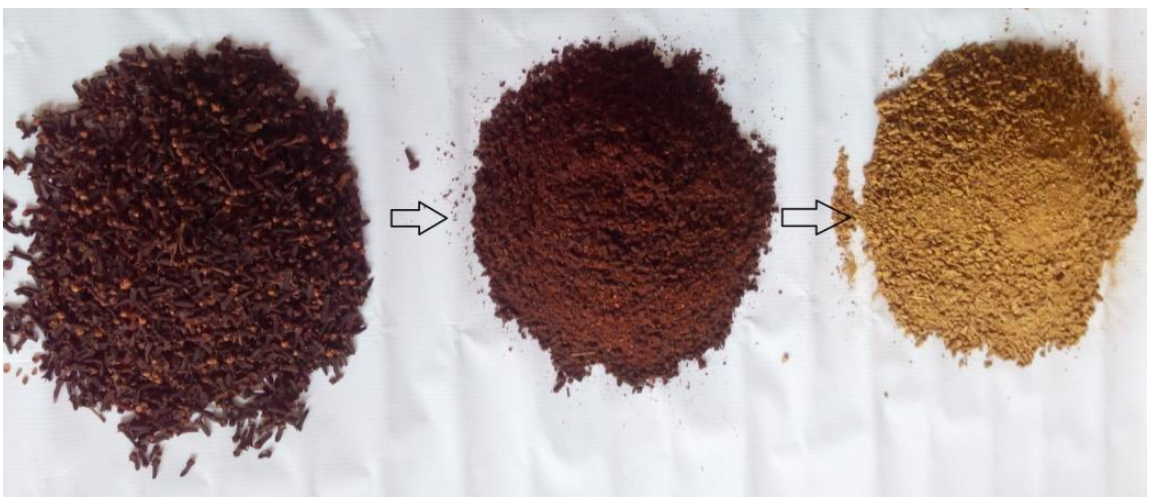


Fig.7.18: Gradual Changes of Colour of Ground Clove Buds before and after SCO_2E

A: (Crude Clove Oil)

B: (Centrifuged Clove Oil)

Fig.7.19 : Clove Buds Oil obtained from Supercritical Carbon-dioxide Extraction

The major chemical constituents of the clove oils obtained from SCO_2E experiments were analyzed using gas chromatograph-mass spectrometry (GCMS), as discussed earlier. The complete GC-MS chromatogram of a clove oil sample is shown in Fig.7.20. The components of clove essential oil were identified by comparing the retention times, mass fragmentation patterns

of them with the available data of reference samples, and GC-MS spectral database for organic compounds. The percent composition of essential oil constituents was determined using computerized normalization method from the peak area of clove oil. The chromatogram of clove oil (Fig.7.20) represents the presence of several bioactive compounds in the clove essential oil. The identified compounds present in the clove oil samples used for quantitative and qualitative analysis in this study were listed in Table 7.10.

The main components identified in the clove extracts were eugenol (58.24-72.08%), eugenyl acetate (11.84-19.28%) and β-caryophyllene (6.73-16.02%). Roughly, the range of these constituents in good quality clove oil was reported as eugenol (70–95 %), eugenyl acetate (up to 20 %) and β-caryophyllene (12–17 %) [Gopalakrishnan, 1988; Jirovetz, 2006; Nurdjannah, 2012].

Eugenol and eugenyl acetate are the main components showing antioxidant activity which is comparable with the activities of both synthetic antioxidants like butylated hydroxyanisole (BHA), pyrogallol [Dorman, 2000] and natural antioxidants like α-tocopherol[Lee, 2001]. Eugenol and β-caryophyllene, these two primary components of clove oil, functions as anti-inflammatory substance [Ghelardini, 2001]. Clove oil is also used as an antimicrobial agent. It was reported that only 0.05% solution of eugenol had the ability to destroy the mycobacterium B. Tuberculosis [Parthasarathy, V. A., 2008]. Clove oil compounds which have the potential to function as anti-cancerous agents are eugenol, β-caryophyllene, β-caryophyllene epoxide, α-humulene, and α-humulene epoxide [Zheng, 1992].

Highest eugenol content (72.08%) was achieved from the extract obtained from optimal condition along with other main components of eugenyl acetate 11.84% and caryophyllene 6.73%. Presence of a larger percentage of main compound eugenol was noticed using annulus extractor bed geometry as compared with the results reported in most of the previous studies. Eugenol content was closed with the results of Huston, K.C., 1991(operating conditions40.53MPa and 40^0C) and Prado, J. M., 2011(operating conditions 15MPa and 40^0C). Thus it can be pointed out that the introduction of annulus geometry inside the extraction vessel improves the quantity of clove oil yield without any compromise with the quality of the product.

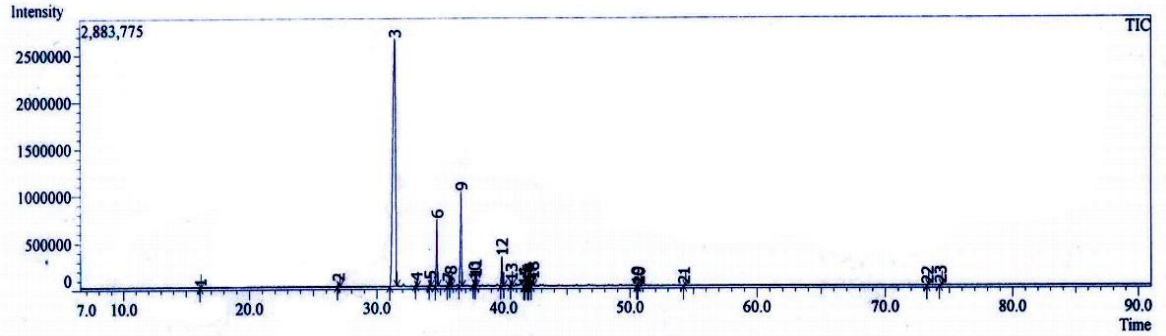


Fig.7.20: Gas chromatogram of the constituents of essential oil extracted from clove buds

Table-7.10: Percentage Chemical Composition of the Clove Buds Oil

Compound Name	Mol. Wt.	Molecular Formula	Retention Time (Min)	% Conc.
P-Allylphenol	134	$C_9H_{10}O$	27.083	0.00-0.44
Eugenol NP	164	$C_{10}H_{12}O_2$	31.441	58.24-72.08
α-Copaene(23.49)	204	$C_{15}H_{24}$	33.106	0.38-0.88
α-Caryophyllene	204	$C_{15}H_{24}$	34.175	0.53-1.82
β-caryophyllene	204	$C_{15}H_{24}$	34.709	8.14-16.02
Humulene-(V1)	204	$C_{15}H_{24}$	35.583	0.00-0.29
Caryophyllene	204	$C_{15}H_{24}$	35.801	0.00-0.88
Eugenyl acetate	206	$C_{12}H_{14}O_3$	36.62	11.84-19.28
Calamenene(Trans) 29.96	202	$C_{15}H_{22}$	37.667	0.52-0.73
Delta-Cadinene	204	$C_{15}H_{24}$	37.757	1.00-0.52
Caryophyllenoxide	220	$C_{15}H_{24}O$	39.845	0.74-3.06
Humulene Epoxide	220	$C_{15}H_{24}O$	40.591	0.00-0.38
Cembrene	272	$C_{20}H_{32}$	41.888	0.00-0.30
2′,3′,4′ Trimethanoxyacetophenone	210	$C_{11}H_{14}O_4$	42.043	0.00-0.49

Rest components were present in the range of 0.03 - 0.24.

7.1.6 Dynamic Mathematical Model of OECs

The OECs obtained from different experiments for RSM studies were found to fit in the Luo Denglin dynamic model type equation [Luo, D. L., 2013]. The model was expressed as-

$$Y = Y_{\infty}\left[1 - \exp(-kt)\right] \qquad \text{----------(7.2)}$$

where Y means the amount of oil extracted at time t. It is expressed as %OY;

Y_{∞} is a measure of the maximum value of Y after infinite time.

k is a rate constant.

The rate constant, k, was expressed as a function of reduced temperature $\left(\frac{T}{T_c}\right)$ and reduced pressure $\left(\frac{P}{P_c}\right)$.

It was defined as – $$\left[k = A \times \left(\frac{T}{T_c} \times \frac{P}{P_c}\right)\right] \quad \text{----------(7.3)}$$

where A is proportionality constant;

T_C = Critical temperature of solvent CO_2 = 31.1^0C and

P_C = Critical pressure of solvent CO_2 = 7.39MPa

Substituting the expression of 'k' from equation (7.3) in equation (7.2), the kinetic model equation takes the form-

$$Y = Y_\infty \times \left[1 - \exp\left(-A \times \left(\frac{T}{T_c} \times \frac{P}{P_c} \times t\right)\right)\right] \quad \text{----------(7.4)}$$

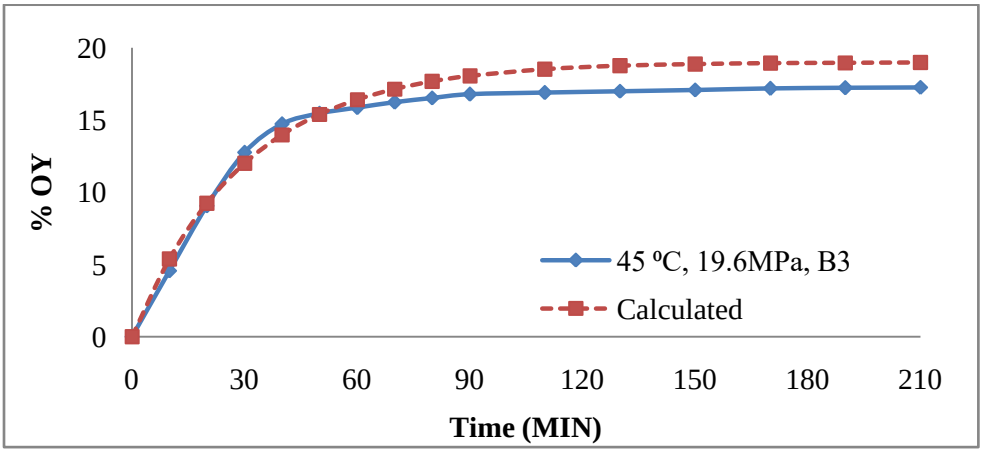
'Y_∞' and 'A' are two model parameters. Y_∞ was substituted from the global yield data obtained from Soxhlet experiment of clove sample. The other model parameter 'A' was estimated analysing the data concerning the overall extraction curves with the help of software Curve Expert 1.40.

The highest yield of clove extract obtained in the Soxhlet process was 19%. It was defined as the global yield. Hence, the value of Y_∞ was replaced by 19. The value of the proportionality constant 'A' was evaluated as 0.012. The dynamic model equation of the overall kinetic extraction was thus found to take the form:

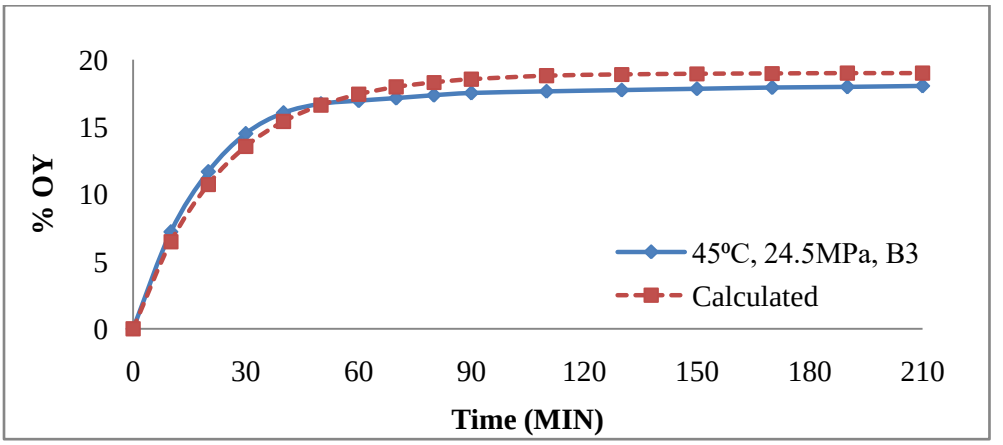
$$Y = 19 \times \left[1 - \exp\left(-0.012 \times \left(\frac{T}{T_c} \times \frac{P}{P_c} \times t\right)\right)\right] \quad \text{----------(7.5)}$$

It described the OECs obtained from SFE of ground clove buds quite well (with R^2 ranges from 0.9-0.99).

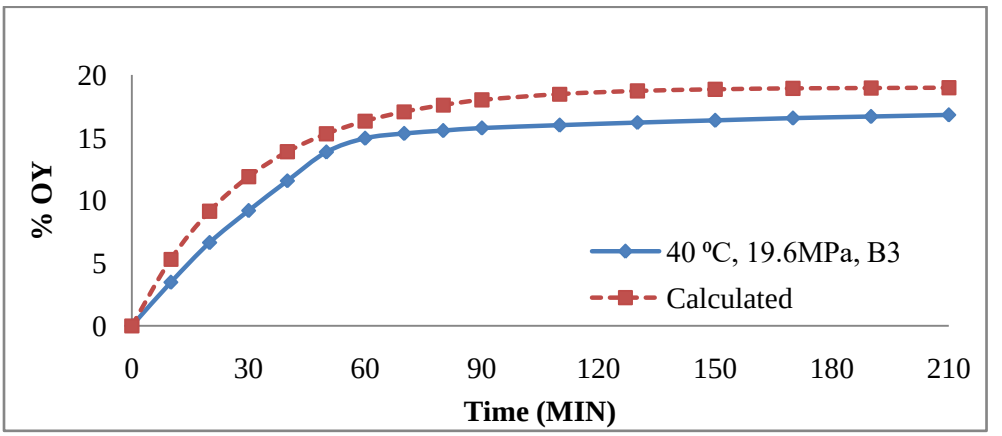
Fig.7.21(a-c) represent model fitting curves and their corresponding actual OECs of clove oil. The operating conditions of extraction pressure and temperature and optimum bed geometry (B3) are provided in the figure. Comparing the actual OECs and model fitting curves, it was noticed that this predicted kinetic model fits well for higher level of extraction temperature and pressure and lower value of ARSEF.



(a)



(b)



(c)

Fig. 7.21: Experimental and Predicted Overall Extraction Curves for Clove Buds under different experimental conditions used in annulus extractor bed B3: (a) 45^0C, 19.6MPa; (b) 45^0C, 24.5MPa; (c) 40^0C, 19.6MPa.

7.2 Outcomes of Turmeric Oil Extraction Experiments

7.2.1 Moisture Content, Particle Size, and Global Yield

The moisture content of dried comminuted turmeric rhizome sample was 12.34%. The yield of extraction and the selectivity of certain bioactive components in solvent CO_2 are influenced positively or negatively by the presence of moisture content in solid plant materials. Ivanovic (2011) reported that the moisture level of plant material between 3-12% has no negative impact on SFE performance for most of the plant matrices. An operational problem was also faced with the presence of high moisture content in the sample biomass in the large scale operation. It arises from the co-extraction of water from the plant material that produces a water-oil mix yield [Prado, 2011] and creates difficulty in developing accurate data set required for plotting OECs. Hence to reduce the co-extraction of water, the turmeric rhizome sample was dried before carrying out any extraction experiment. The moisture content of the dried turmeric sample was 4.29%.

The ground moisture treated turmeric sample was classified into three different particle sizes of 0.3mm, 0.6mm, and 0.9mm that were used later in the extraction experiments. The total extractable matter or the global yield of ground turmeric sample that was determined in the Soxhlet Apparatus to use as reference yield in future SFE study was 5.42%. The result of the Soxhlet extraction of turmeric rhizome was found to agree well with some available works of literature [Braga, 2003; Priyanka, 2018]. These results are provided in Table-7.11.

Table-7.11: Moisture Content, Particle Size and Global yield of Turmeric Sample

Moisture content of raw Turmeric Rhizome (wt %)	Moisture content of dry milled Turmeric Rhizome (wt %)	Particle Size			Global Yield of Turmeric Rhizome Oil (%OY)
		DP1(mm)	DP2 (mm)	DP3 (mm)	
12.34	4.29	0.3	0.6	0.9	5.42

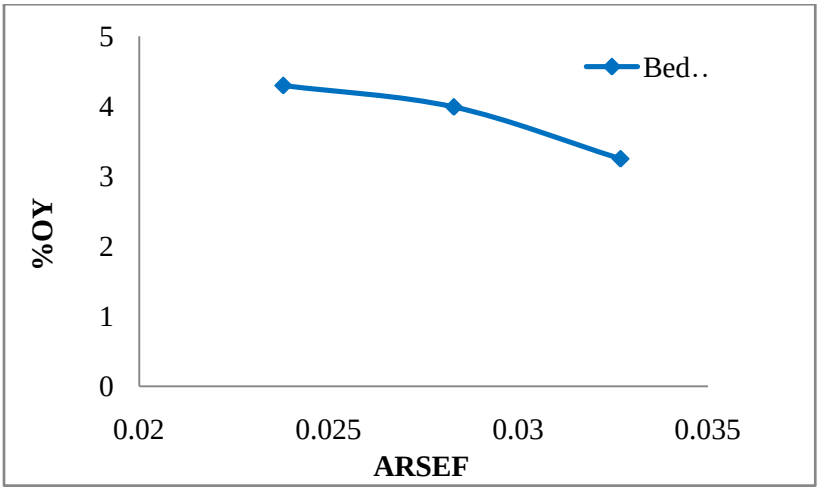
7.2.2 Effect of Extractor Bed Geometry on SCO_2E Yield of Turmeric Oil

The results of SFE studies on clove buds (section 7.1) show that the geometric modification of the extraction vessel from conventional cylindrical geometry to annulus geometry attributed successfully to the extractor performance in terms of improving the rate of extraction and yield of clove oil in a considerable amount. In this section to study the SFE of turmeric rhizomes, the bed geometry of extraction vessel was chosen as a primary factor to find out its effect on turmeric oil recovery from its rhizomes using supercritical solvent CO_2. The pressure and temperature of extraction applied for bed geometry study were 24.5MPa and 50^0C, respectively. All other operation parameters were kept constant. Experimental results of extraction time and corresponding yield of turmeric oil obtained applying different bed geometry were provided in Table7.12.

Table-7.12: Extraction Time vs. Yield Data of Turmeric for Different Bed Geometry

Extraction Time (Min)	B1	B2	B3
	% OY	% OY	% OY
0	0.00	0.00	0.00
15	0.50	0.79	1.02
30	0.92	1.47	1.95
45	1.38	2.14	2.75
60	1.75	2.50	3.12
90	2.15	2.85	3.41
120	2.40	3.04	3.62
150	2.55	3.31	3.75
180	2.83	3.48	3.98
210	3.07	3.79	4.14
240	3.25	3.99	4.30

Variation in bed geometry of the extractor affects the overall extraction curves (OEC) and contributes significantly on extraction kinetics, the quantity of yield, quality of extract, and cost involved with the extraction process. The impact of the geometry of the extraction vessel on the extractable amount of oil is graphically represented with the help of %OY vs. ARSEF plot (Fig.7.22). ARSEF was defined to represent the bed geometry configurations previously. The maximum value of ARSEF represents common type cylindrical geometry (B1) and the other two smaller values of ARSEF correspond to two different annulus geometries (B2 and B3) of the extraction vessel.



ARSEF=0.0327 (B1), ARSEF=0.0283 (B2), ARSEF=0.0238 (B3)

Fig.7.22: Effect of Annulus and Cylindrical Bed Geometry on Yield of Turmeric Rhizome

The nature of the plot of Fig.7.22 demonstrates that the dimensionless group ARSEF representing bed geometry and %OY of turmeric are inversely related to each other. It reveals that under the same operating conditions, the yield of turmeric oil was raised more when cylindrical bed geometry was replaced by annular bed geometry comparing with the same due to the interchange of two different annulus geometry. The highest yield of 4.3% was pointed out in case of annulus extractor vessel with larger annular channel (B3) as compared to yield of 3.99% in case of annulus extractor vessel with smaller annular channel (B2) and lowest yield of 3.25% (which was 20% lower than the yield of B3 with respect to global yield) in case of common type cylindrical extractor without an annular channel (B1). Almost 80% of global yield obtained from Soxhlet extraction was recovered in this study using annulus extractor bed geometry (B3) without application of any co-solvent for an extraction period of 240min and operating conditions of 50^0C temperature and 24.5MPa pressure. These results of yields obtained from fully loaded extraction vessel (1L) without applying any glass ball for uniform solvent distribution are comparable with that reported by Gopalan (2000) and Priyanka (2018).

The relation among the variables of extraction time, bed geometry of extraction vessel and extraction yield is shown in Figure 7.23. This plot clearly described the effect of bed geometry on the rate of extraction as well as the final yield for the same period of extraction by graphical means. The yield of turmeric oil increased with increasing extraction time despite the type of bed geometry until the equilibrium was established. The graphs showed that the rate of extraction for both types of annulus bed geometry (B2 and B3) was faster compared to the cylindrical geometry (B1).

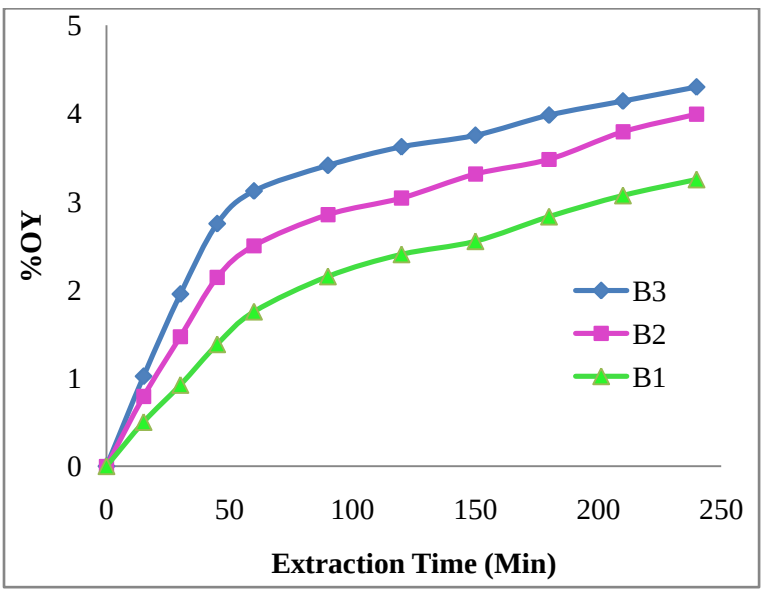


Fig.7.23: Effect of Extractor Bed Geometry and Extraction Time on Yield of Turmeric Rhizome

For annulus bed geometries the slopes of the first portion of the OECs were much steeper compared to the slope of cylindrical geometry. This initial faster extraction period where the main mass transfer occurs at the surface of the molecules is the most crucial stage of extraction as it directs the process to achieve the targeted recovery level (generally 90% of the extractable

quantity) in shorter period and thus, reduce the operational cost of extended extraction period [Valle, 2005]. The faster rate of mass transfer between solid-fluid phases can be achieved by improved interaction between the phases, induced rate of diffusion, and faster transport of the diffused molecules. In the present study of turmeric oil extraction by SCO_2, the introduction of annulus bed arrangement replacing the cylindrical geometry inside the extractor exhibits a decisive role on the process within its design range as observed in the case of clove oil extraction in the previous section. It was resulted from the modification of radial surface area ($2\pi L(r_o+ r_i)$) and axial surface area ($\pi(r_o^2-r_i^2)$) of the extraction vessel raised from annulus geometry which reduces the molecular diffusive path for all the molecules and induces turbulence in the bulk fluid that improves the solid-fluid interaction as well as convective diffusion. Thus, the mass and heat transfer throughout the extraction span favoured the recovery of turmeric oil in a much better way and produced higher order extract in case of annulus extractor as compared to the cylindrical extractor. At the same time, while detail design of the annulus bed extractor should be developed the detrimental effect of larger voids on channelling must come under consideration.

7.2.3 Optimization of SCO_2 Extraction Process for Extraction of Turmeric Oil

The process optimization experiments of turmeric oil extraction from dried comminuted rhizomes were conducted in the same semi-continuous supercritical fluid extraction equipment (Model No: CSL/SCF/1L2/400) used in the study of clove oil extraction optimization. To perform the SFE experiments at various operating conditions of the selected factors as suggested in FC-CCD design, selecting one suitable extractor geometry from B1, B2 and B3 was a mandatory requirement. Annulus feed bed B3 among these three types was selected as the optimal geometric condition within present design limits as it extracted turmeric oil as well as clove oil most efficiently using a supercritical solvent in the previous runs. Three factors which were chosen to investigate their direct/indirect and independent/interactive role on extraction process to maximize the yield of turmeric oil were pressure (A1), temperature (A2), and particle size (A3). These factors were verified at their three levels to optimize the extraction process to improve the response (or yield of turmeric oil) by RSM developed under statistical analysis.

The values of operating parameters and responses of SFE experiments conducted under optimization study are provided in Table-7.13. The quadratic model was observed to fit the responses (%OY) with the operating parameters well as compare to linear and two-factor interaction (2FI) models based on R^2, standard deviation, adjusted R^2, predicted R^2, "PRESS" values, F-values, p-values, and lack-of-fit tests results. Larger F-value (25.69), negligible p-value <0.0001, low value of standard deviation (0.0256), high value of R^2 (0.9992), lowest "PRESS" value and larger adjusted R^2 (0.9984) and largest predicted R^2 (0.994), and (Adjusted R^2 - Predicted R^2) < 0.2 suggested that the quadratic model showed insignificant lack of fit for all the input operating factors and response data of yield.

In the next step, an ANOVA test was performed to provide statistical information about the significant fitting of each linear, FI, and quadratic terms from their individual P-value. Lower P-values below 0.05 for most of the terms indicate that they were all essential terms of the final model equation. High model F-value of 1345.94 satisfies the significance of the quadratic model equation. ANOVA test results are illustrated in Table-7.14.

Table -7.13: FC-CCD data of three independent variables with their coded levels and response as percentage oil yield (% OY) of Turmeric Rhizome using SCO2 Extraction

Run No.	Input Factors			Response
	A1, Pressure (MPa)	A2, Temperature (^{0}C)	A3, Particle Size (mm)	%OY (gm oil/100 gm Feed)
1	24.5 (0)	40(-1)	0.6(0)	3.25
2	21.6 (-1)	40(-1)	0.3(-1)	3.76
3	24.5 (0)	50(0)	0.6(0)	3.51
4	24.5 (0)	50(0)	0.6(0)	3.50
5	27.5 (+1)	50(0)	0.6(0)	3.74
6	24.5 (0)	50(0)	0.6(0)	3.50
7	24.5 (0)	50(0)	0.6(0)	3.52
8	21.6 (-1)	60(+1)	0.3(-1)	4.30
9	27.5 (+1)	60(+1)	0.9(+1)	3.20
10	21.6 (-1)	60(+1)	0.9(+1)	2.70
11	27.5 (+1)	60(+1)	0.3(-1)	4.45
12	24.5 (0)	50(0)	0.6(0)	3.50
13	27.5 (+1)	40(-1)	0.9(+1)	2.65
14	27.5 (+1)	40(-1)	0.3(-1)	4.23
15	24.5 (0)	50(0)	0.3(-1)	4.30
16	21.6 (-1)	50(0)	0.6(0)	3.23
17	24.5 (0)	50(0)	0.9(+1)	2.62
18	24.5 (0)	60(+1)	0.6(0)	3.72
19	21.6 (-1)	40(-1)	0.9(+1)	1.84
20	24.5 (0)	50(0)	0.6(0)	3.50

Table-7.14: ANOVA for Suggested Model to analyze parametric effects on Turmeric Oil

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.95	9	0.8836	1348.94	< 0.0001	significant
A1, Pressure	0.5954	1	0.5954	908.85	< 0.0001	
A2, Temperature	0.697	1	0.697	1063.95	< 0.0001	
A3, Particle Size	6.45	1	6.45	9843.39	< 0.0001	
A1A2	0.0496	1	0.0496	75.74	< 0.0001	
A1A3	0.0595	1	0.0595	90.85	< 0.0001	
A2A3	0.0528	1	0.0528	80.62	< 0.0001	
$A1^2$	0.0026	1	0.0026	3.89	0.0767	
$A2^2$	0.0026	1	0.0026	3.89	0.0767	
$A3^2$	0.0085	1	0.0085	12.91	0.0049	
Residual	0.0066	10	0.0007			
Lack of Fit	0.0062	5	0.0012	17.72	0.0034	significant
Pure Error	0.0003	5	0.0001			
Cor Total	7.96	19				
Std. Dev.	0.0256					
R^2	0.9992					
Adjusted R^2	0.9984					
Predicted R^2	0.994					
Adeq. Precision	144.8787					

Here A1, A2, and A3 relate the effects of primary process parameters pressure (MPa), temperature (^{0}C), and particle size (mm) on the response (%OY). $A1^2$, $A2^2$, and $A3^2$ produce the quadratic effects of the same input variables. A1A2, A1A3, and A2A3 express the interaction effects of three possible combinations of three factors (i) pressure and temperature; (ii) pressure and particle size, and (iii) temperature and particle size, respectively.

7.2.3.1 Model Equation obtained from RSM

The final form of the quadratic mathematical model equation that expresses the yield of turmeric oil (% OY) as a function of the three optimizing process variables of RSM study within their design range is given by the following generalised formula:

$$\%OY = \beta_0 + \beta_1 A1 + \beta_2 A2 + \beta_3 A3 + \beta_{12} A1A2 + \beta_{13} A1A3 + \beta_{23} A2A3 + \beta_{11} A1^2 + \beta_{22} A2^2 + \beta_{33} A3^2$$

where %OY is the actual response;

A1, A2, and A3 are the un-coded variables extraction pressure, temperature and particle size, respectively;

β_0 is the regression coefficient of intercept;

β_1, β_2, and β_3 are the regression coefficients for linear fit;

β_{12}, β_{13}, and β_{23} are the regression coefficients for FI fit (i.e., pressure-temperature, pressure- particle size and temperature-particle size interactive factors); and

β_{11}, β_{22}, and β_{33} are the regression coefficients for quadratic fit.

The actual values of the regression coefficients as evaluated from the ANOVA test are given in Table-7.15.

Table-7.15: Coefficients of Predictive Quadratic Model obtained from ANOVA for Turmeric Oil

	β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{11}^2	β_{22}^2	β_{33}^2
%OY	3.50918	0.244	0.264	-0.803	-0.07875	0.08625	0.08125	-0.03045	-0.03045	-0.05545

This equation in terms of actual operation parameters with evaluated regression coefficients is useful to make predictions about the yield (%OY) of turmeric oil extraction using SCO_2. The value of each factor (A1, A2, and A3) within their specified level should be substituted in their original units.

Actual responses of all experiments performed during optimization study and corresponding predicted responses evaluated using the final quadratic equation are provided in Table-7.16 (a) & (b). These results are graphically shown in Fig. 7.24.

Table-7.16(a): Actual Value of %OY and Predicted Value of %OY as per ANOVA

Run Order	1	2	3	4	5	6	7	8	9	10
Actual Value of %OY	3.25	3.76	3.51	3.50	3.74	3.50	3.52	4.3	3.2	2.7
Predicted Value of %OY	3.21	3.78	3.51	3.51	3.72	3.51	3.51	4.30	3.19	2.68

Table-7.16 (b): Actual Value of %OY and Predicted Value of %OY as per ANOVA

Run Order	11	12	13	14	15	16	17	18	19	20
Actual Value of %OY	4.45	3.50	2.65	4.23	4.30	3.23	2.62	3.72	1.84	3.50
Predicted Value of %OY	4.46	3.51	3.65	4.25	4.26	3.23	2.65	3.74	1.84	3.51

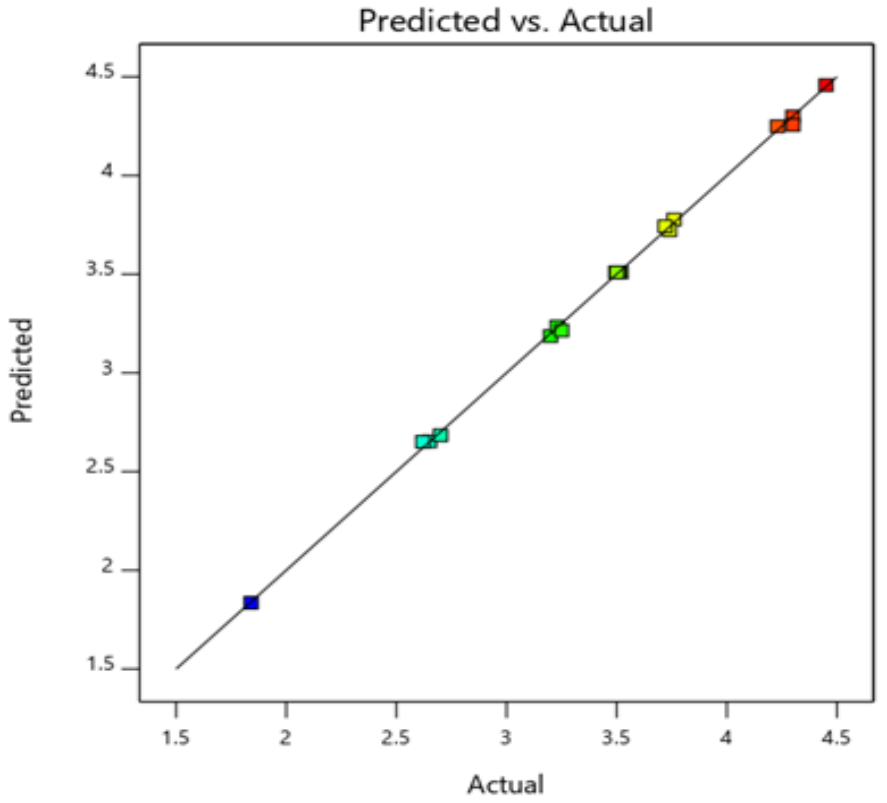
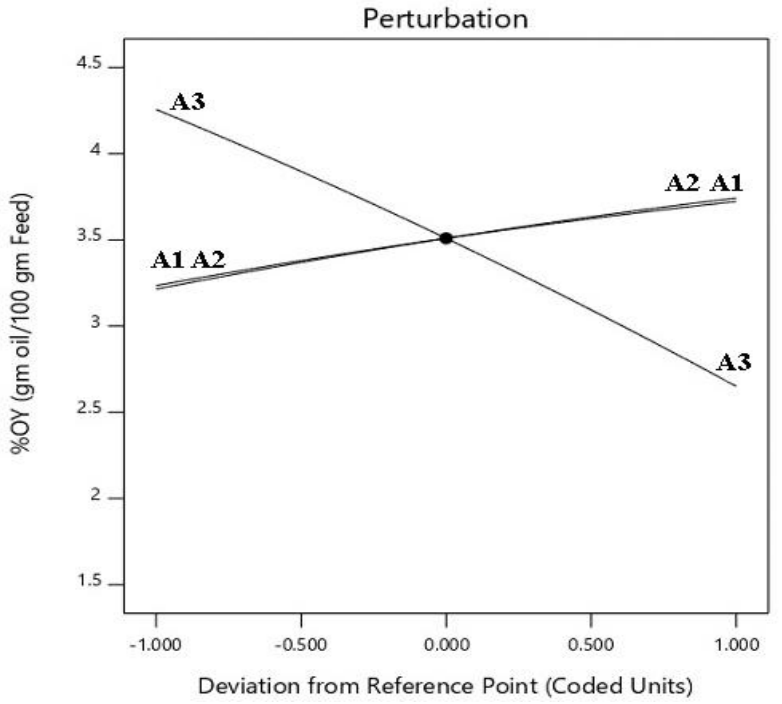


Fig.7.24: Graphical Representation of Predicted Response vs. Actual Response of %OY of Turmeric

7.2.3.2 Effects of Extraction Pressure, Temperature and Particle Size on Turmeric Oil Yield

Fig 7.25 graphically represents the response (yield of turmeric oil, %OY) vs. all operating parameters (pressure, temperature and particle size) chosen in this optimization study in a single response plot known as perturbation plot. It describes the variation of yield with changing one of the chosen extraction parameters over its design range while all the other factors remain unaltered. The nature of dependency of the extraction yield on a particular parameter to be optimized is relatively understood from the nature of slope or curvature. In some cases, the most influential factor of extraction from the chosen parameters is also possible to identify with the help of perturbation plot.



A1 : Extraction Pressure (MPa), A2 : Extraction Temperature (^{0}C), A3 : Particle Size (mm)

Fig.7.25: Perturbation Plot to explain the effects of SFE Optimizing Parameters on %OY of Turmeric Oil

❖ **Influence of Extraction Pressure**

Extraction pressure (A1) is a crucial parameter that influences the extraction efficiency by its excellent tuning effects on extraction properties like density, solubility, selectivity, and viscosity of the supercritical solvent. For investigating the effect of extraction pressure on the recovery of turmeric oil, the dried milled turmeric rhizomes of various sizes were extracted at different temperature levels as suggested by FC-CCD at three different pressures of 21.6, 24.5and 27.5MPa. The nature of the %OY vs. A1 (pressure) graph in the perturbation plot (Fig. 7.25) indicates that increasing pressure throughout the range selected for optimization favours the turmeric yield recovery. As operation pressure was gradually increased from 21.6MPa to 27.5MPa, the average yield of turmeric also increased from 3.17% to 3.65% for different extraction temperatures and particle sizes. The highest extraction yield (4.45%) of turmeric rhizomes was obtained at the highest pressure level of 27.5MPa. It shows that more than 80% of extractable oil was possible to extract using annulus bed geometry without applying any co-solvent at moderate temperature and pressure. It resulted from the pressure-density and density-solubility proportional relationship. Increasing pressure above critical value caused a tremendous rise of solvent density, enhancing the solvent-solute interaction and solubility of oil molecules significantly that raised the rate of oil recovery as well as final yield overcoming the adverse effect of increased viscosity and reduced diffusivity [Liza, 2010]. Thus it can be concluded that the introduction of annulus bed geometry may not affect the normal behaviour of pressure on oil recovery from plant matrix.

Effect of extraction pressure and extraction time on the rate of mass transfer and yield of turmeric oil is possible to explain with the help of OECs (Fig. 7.26) generated from the experimental results of SFE runs conducted in B3 type extractor at 50^0C temperature and different levels of pressure using ground turmeric of particle size 0.6mm. It is reflected from the graphs that with increasing time, the yield of turmeric oil increased for all the runs until the equilibrium was established. On the other hand, with increasing pressure, the slope of extraction curve slightly increases that was indicative of the faster rate of extraction due to increase in solubility and final rise in productivity of turmeric oil (3.23 % for 21.6MPa, 3.51% for 24.5MPa and 3.74% for 27.5MPa) [Priyanka, 2018].

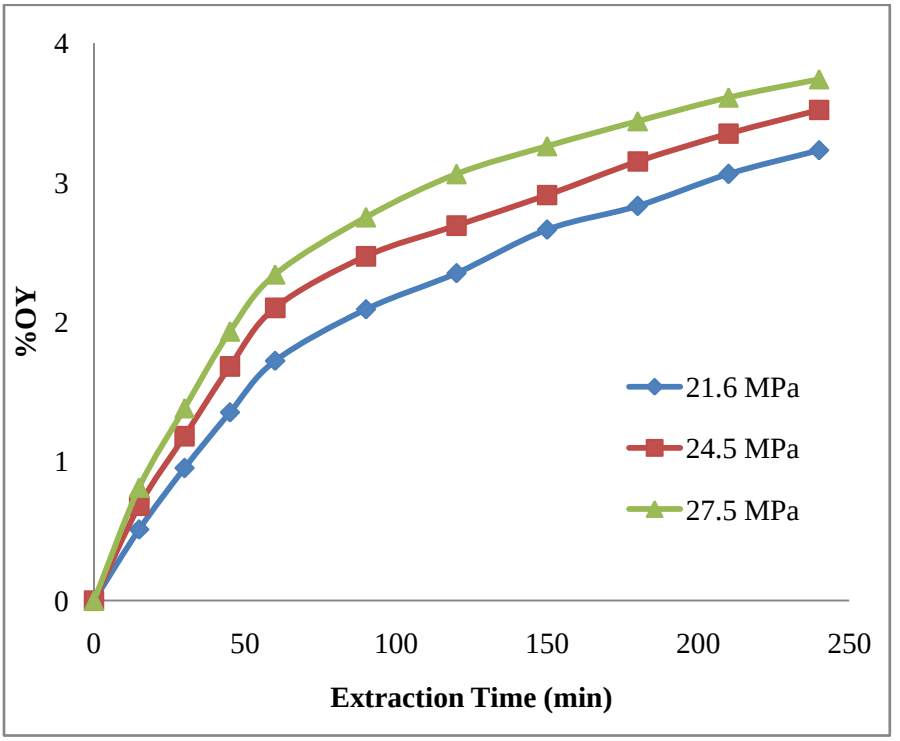


Fig.7.26: Effect of Pressure and Extraction Time on Yield (%OY) of Turmeric (0.6mm size) at 50^0C

❖ **Influence of Extraction Temperature**

Like extraction pressure, extraction temperature also shows a direct influence on density, viscosity, vapour pressure and diffusivity parameters. As discussed earlier (section 7.1.2) with increasing temperature above the critical point, density and density-dependent property, solubility of solute, decrease; vapour pressure of solute and hence solubility of solute increases; diffusion coefficient of mass transfer increases; and viscosity of solvent also decreases. Reduced viscosity promotes the penetration of the fluid phase through the ground particles. Increased vapour pressure and diffusivity while contribute to the rate of mass transfer faster, the negative effect of density on solubility inhibits it [Terada, 2010; Rai, 2015]. In this study the nature of the %OY vs A2 (extraction temperature) graph in the perturbation plot (Fig. 7.25) indicated that in the temperature range from 40-60^0C,while temperature was raised the average yield of oil increased

from 3.15% to 3.67% despite the negative impact of reduced density on solubility that was diminished by solubility inducing another factor, vapour pressure, whose effect became more prominent in this temperature range.

Fig. 7.27 validates the positive impact of temperature on extraction rate and oil yield in a more precise way with the help of OECs developed from FC-CCD experiments conducted at 24.5MPa pressure in B3 type extractor using ground turmeric sample of 0.6mm size. It is noticed from the graphs that with increasing temperature the slope of extraction curves increases slightly (similar way like pressure) that results in an increase of final yield of turmeric oil (3.25 % for 40^0C, 3.51% for 50^0Cand 3.72% for 60^0C). Temperature behaviour of the present study confirmed the results of Carvalho (2015), and Priyanka (2018), whereas contradicted the results of Chang (2006) and Gopalan. (2000). Variations in the nature of binding of the oil molecules with the solid matrix are considered as one of the probable reason such contradicting effect of temperature.

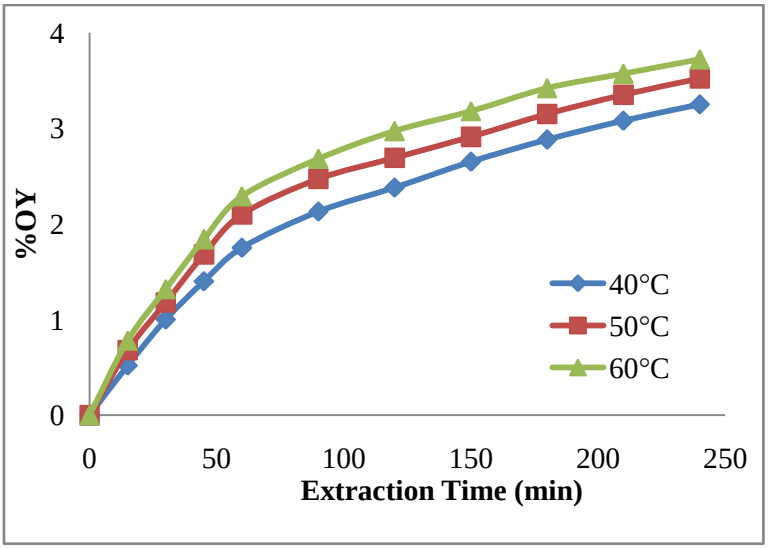


Fig.7.27: Effect of Temperature and Extraction Time on Yield of Turmeric (0.6mm size) at 24.5MPa

❖ **Influence of Extraction Particle Size**

The perturbation plot (Fig. 7.25) of the present study indicates that the particle size of ground turmeric rhizomes among three chosen parameters of optimization was the most influential factor for efficient extraction. The results of the statistical analysis also indicated the same. The nature of the perturbation plot (Fig. 7.25) suggests that the particle size (A3) and extraction yield (%OY) of turmeric are negatively correlated with each other. As a result, the turmeric oil extracted using supercritical fluid was increased with decreasing particle size. While the particle size of turmeric sample decreased in the order 0.9mm, 0.6mm to 0.3mm, the average yield of turmeric oil increased as 2.6%, 3.49% to 4.21%, respectively. Ab Rahman (2012) stated in his study that the most crucial parameter that controls the yield of SFE and extraction efficiency is the particle size of the solid matrix. In the study on turmeric rhizome, Priyanka (2018) also observed that particle size is a dominating factor to decide the yield of oil. This is explained as –(i) particles of larger

size provide barrier in internal diffusion of the solvent CO_2 to reach the oil layer of unbroken cells and thus require a longer extraction process and higher solvent flow [Wang, 2006; Ab Rahman, 2012]; (ii) particles of smaller size open up the oil molecules from broken cells to access easily by the solvent [Machmudah, 2007] and provide more interfacial surface area of contact [Pourmortazavi, 2007] that is proportional with the mass transfer coefficients [Stastova, 1996] to improve the yield. In this regard, it needs to be mentioned that very fine powder material may sometimes inhibit the extraction process due to the difficulty faced in maintaining the flow of solvent [Wang, L., 2006] and the slower flow rate was recommended for fine particles [Ab Rahman, 2012]. Chang, L.H. (2006) recommended that use of particles larger than 0.42mm was not beneficial in terms of turmeric yield and Gopalan (2000) reported a gradual increase in turmeric oil for the particle size reduction from 1.158mm to 0.08mm that fitted well with the results of the present study. However, the introduction of annulus bed geometry inside the extraction vessel resulted in the recovery of more oil as compared to the results of Gopalan (2000), particularly for the larger particle (0.9mm).

The effect of size reduction of dried turmeric rhizomes on the rate of mass transfer and final yield of extract for a constant time of extraction is possible to explain in a more precise way with the help of OECs (Fig. 7.28). It is noticed from the graph that with decreasing particle size, there is a sharp change in mass transfer rate (indicated from the increasing slope steepness) and oil recovery. SFE of turmeric rhizomes at 24.5MPa pressure and 50^0C temperature produced an increased yield from 2.62% for 0.9mm particle size to 4.3% for 0.3mm particles for bed geometry B3 which is equivalent to 30% more yield recovery w.r.t. the total extractable oil (5.42%).

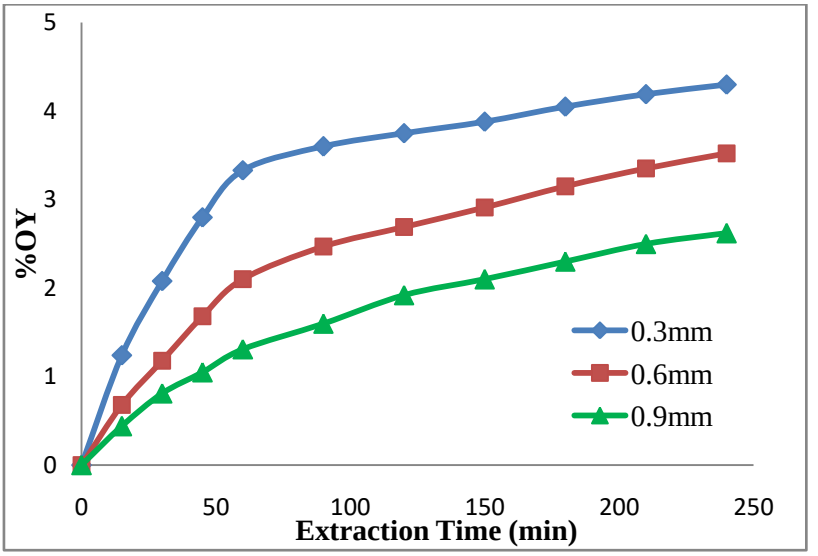


Fig.7.28: Effect of Particle Size and Extraction Time on Yield of Turmeric Oil at 24.5MPa and 50^0C

7.2.3.3 Effects of Two Factors Interaction on Turmeric Oil Yield

The response surface plots are used to explain the effect of any two-factor interaction on the extract of turmeric in the range of values chosen for investigation. These three-dimensional plots describe the nature of dependency on the chosen variables and direction of optimization of the

process. In this section three response surface plots namely, temperature – pressure; temperature – particle size and pressure – particle size, are explained to understand their role on turmeric oil extraction process in a modified annuls extractor bed geometry.

❖ **Interaction effect of Extraction Pressure and Temperature**

In SFE of plant materials selection of suitable pressure and temperature play the crucial roles in selectively separating the oil molecules as these two essential factors directly control the mass separation properties like density, viscosity, solubility, selectivity, and diffusivity near the critical point. Nature of pressure-temperature surface plot (Fig.7.29) indicates that increase of both pressure (in the range of 21.6-27.5 MPa) and temperature (in the range from 40^0C - 60^0C) of extraction exhibit relatively slight positive impact on % recovery of oil [Priyanka, 2018]. At a given particle size, SFE using annulus feed bed improves the yield of turmeric oil with increasing any one factor between extraction pressure and temperature keeping the other constant throughout the chosen levels of these two factors. The highest quantity of yield of turmeric oil corresponds with the highest levels of both factors on the graph at 27.5MPa and 60^0C. The minute increase in oil extract under the influence of the significant rise of both pressure and temperature may be explained with the help of opposing behaviours of both pressure and temperature on the transport process. Increased yield with increased process parameters means that pressure and temperature of the extraction function together to improve the solute-solvent interaction, vapour pressure of soluble oil, solubility, and diffusivity of molecules in the medium. Whereas the increase of yield in small degree indicates that strong adverse effects of temperature rise (on solubility by density reduction) and pressure rise (on solvent penetration due to increased viscosity and rate of extraction by reduced diffusivity) diminished the favourable mass transport properties to some degree.

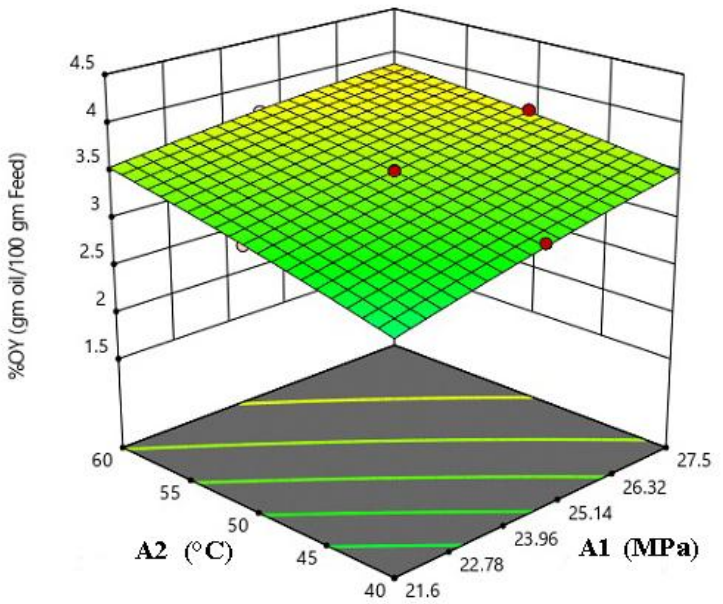
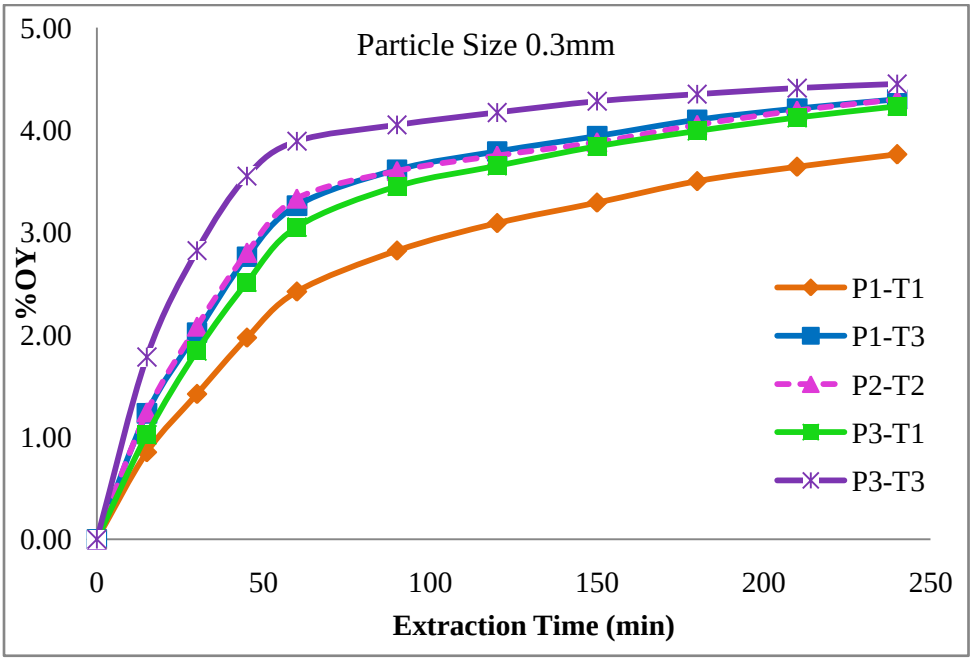


Fig.7.29: Pressure (A1) –Temperature (A2) Interaction Effect on Extraction Yield

Fig 7.30-7.32 are the OECs that represent the pressure-temperature interaction effects on mass transfer rate and yield of turmeric oil for given particle size. For the smallest size particle (0.3mm), it is observed that the highest and lowest temperature-pressure levels influence the rate of extraction as per their trends (Fig 7.30). All other temperature-pressure settings shown in the graph influence the rate of oil recovery almost in a similar way with minor variations.

For intermediate particles of 0.6mm size, highest pressure or highest temperature settings provide faster rate and produce yields of higher-order and with reducing pressure or temperature gradually from intermediate to lower level, rates of mass transfer drop and consequently yields of oil decrease.

For largest particle size(0.9mm) pressure-temperature interaction behaviours (Fig.7.32) follow symmetric relation like that observed in case of smallest size particle (Fig7.30),only with a significant drop in oil recovery.



P1=21.6MPa, P2=24.5MPa, P3=27.5MPa; T1=40^{0}C, T2=50^{0}C, T3=60^{0}C

Fig.7.30: Pressure – Temperature Interaction Effect on OECs for 0.3mm Turmeric Particle

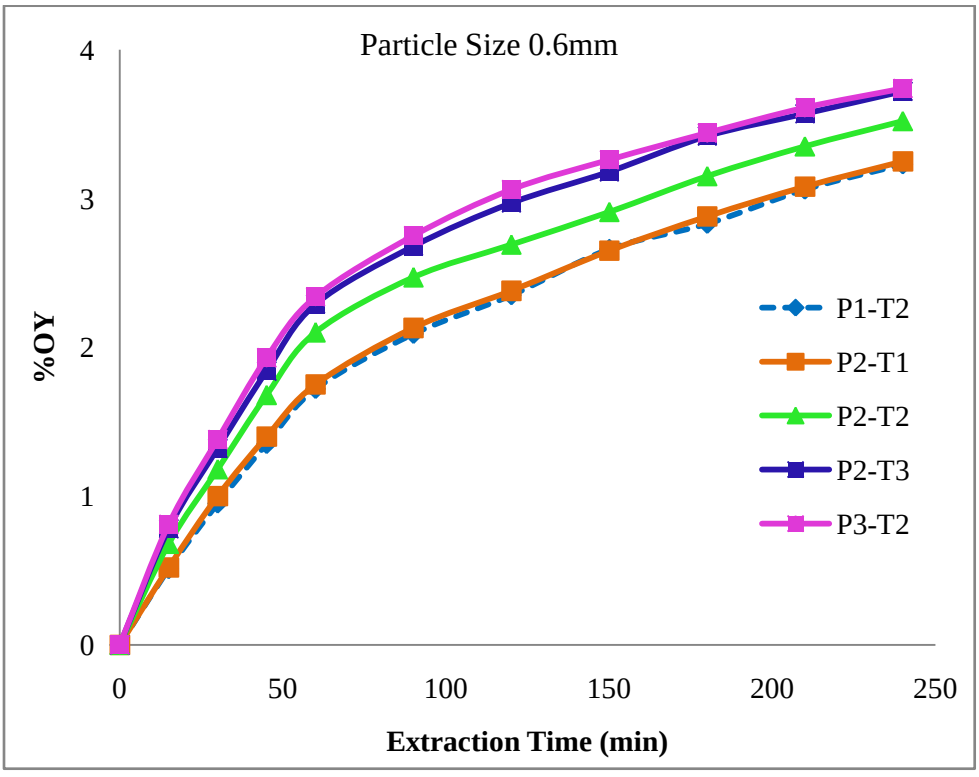


Fig.7.31: Pressure – Temperature Interaction Effect on OECs for 0.6mm Turmeric Particle

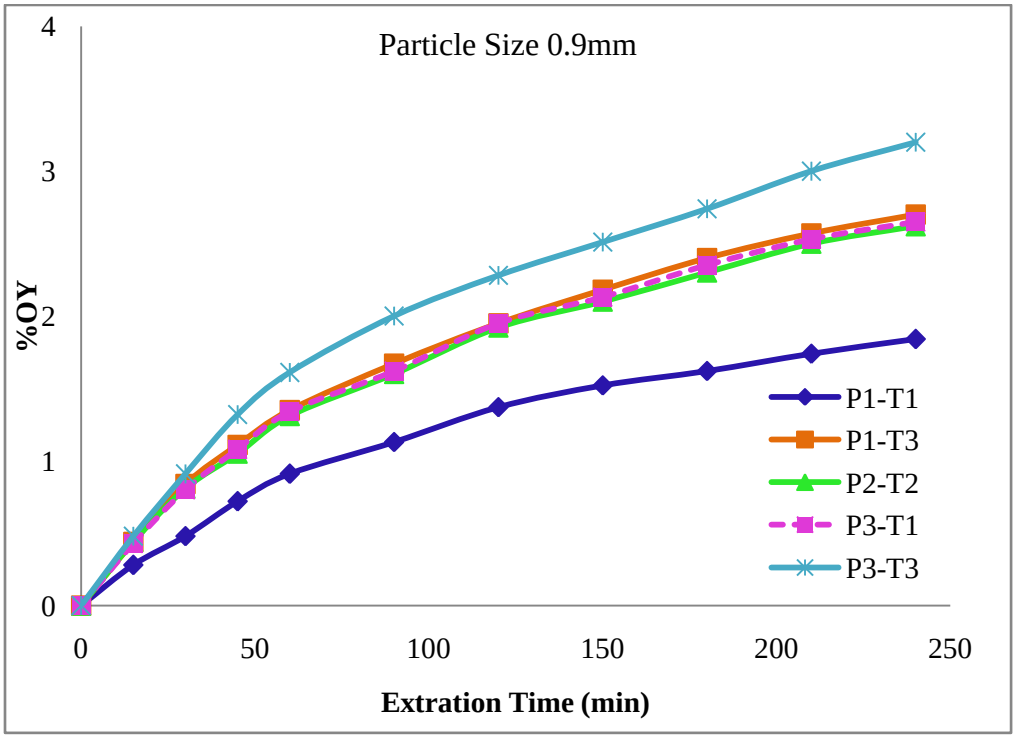


Fig.7.32: Pressure – Temperature Interaction Effect on OECs for 0.9mm Turmeric Particle

❖ **Interaction effect of Extraction Pressure and Particle Size of Turmeric Rhizomes**

Fig.7.33 represents the interaction effect of both extraction pressure and particle size of turmeric rhizomes on the yield of supercritical fluid extraction performed in the optimization study. The nature of the surface plot indicates that pressure-particle size interaction shows a strong influence on the yield of turmeric oil within design values. At a given temperature when ground turmeric samples were extracted in annulus extraction vessel, it was observed that increase of extraction pressure from 21.6 (P1) to 27.5MPa (P3) caused higher recovery of oil for a particular particle size with higher order recovery in case of smaller size particle (DP1). The maximum amount of turmeric oil has resulted from the highest level of pressure (P3=27.5MPa) and the lowest value of particle size (DP1=0.3mm) for a constant temperature. It is because of the improvement in the solubility property of the oil and solute-solvent interaction under the influence of high pressure and increase of the damaged cells due to size reduction that improves the interfacial area of contact between the phases and makes the oil molecules easily accessible by reduction of internal diffusion path.

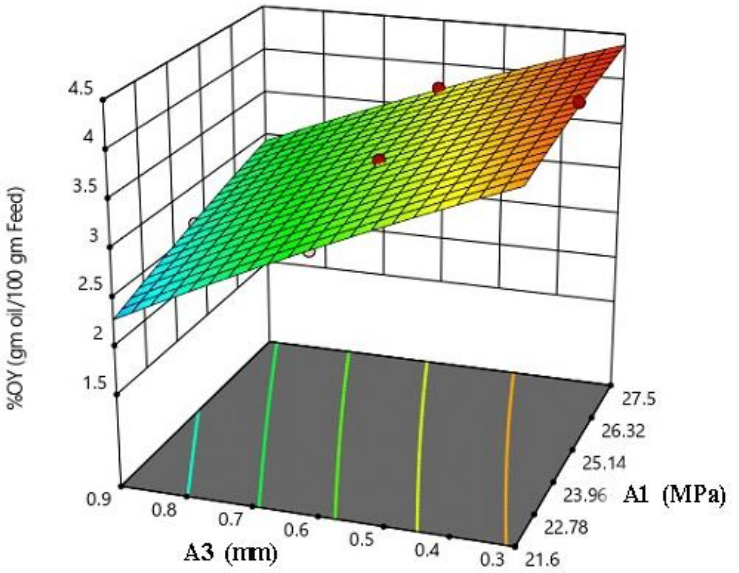


Fig.7.33: Pressure(A1)– Particle Size (A3) Interaction Effect on Extraction Yield

Fig. 7.34 graphically shows the interaction effects of particle size and extraction pressure on the rate of extraction of turmeric oil that affects the OECs. At highest operating temperature, SFE using annulus extraction vessel produced yields with sufficiently larger quantity for smallest size particles (P3-DP1, and P1-DP1) compared with largest size particles for same levels of pressure (P3-DP3 and P1-DP3). Thus it can be observed that the adverse effect of larger size particles on the rate of extraction and final recovery of oil is very strong. The undamaged cells of larger particles provide tremendous resistance in the internal diffusion to extract oils that are not even possible to overcome by applying elevated pressure. It shows that yield of oil produced at P1-DP1 combination was even higher than the yield at P2-DP2 setting at an elevated temperature of 60^0C.

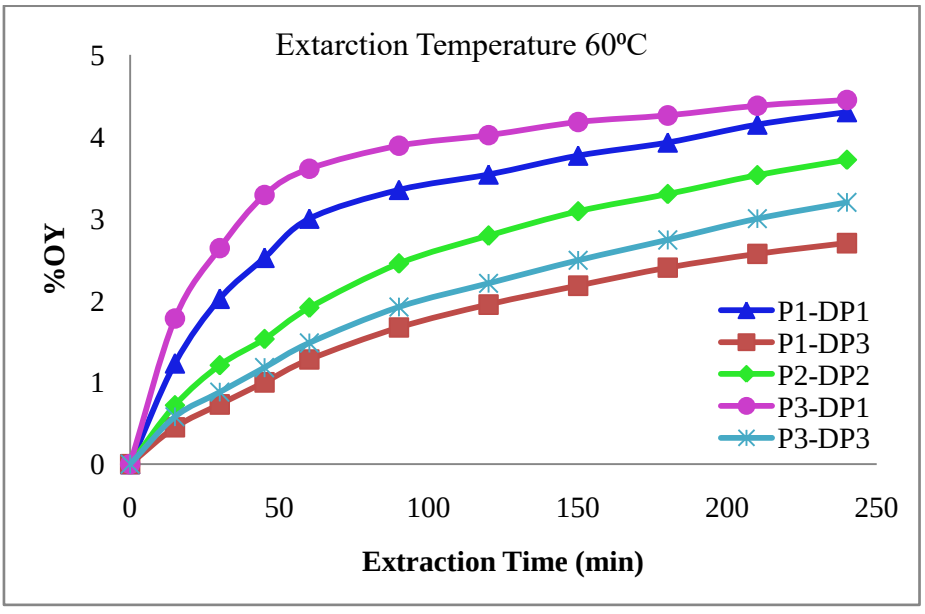


Fig.7.34 : Pressure – Particle Size Interaction Effect on Overall Extraction Curves of Turmeric oil

❖ **Interaction effect of Extraction Temperature and Particle Size of Turmeric Rhizomes**

Fig.7.35 represents the interaction effect between extraction temperature and particle size of ground turmeric on the supercritical fluid extraction yield. The response surface plot shows that extraction temperature-particle size interaction also influences the yield of turmeric oil strongly. During analyzing the surface plot, it was pointed out that increasing temperature and decreasing particle size within their design range are suitable to improve the yield recovery. At any given temperature within 40-60^{0}C, the trend of increasing yield has resulted from reduced size particles of turmeric sample. Highest temperature (60^{0}C) and smallest size (0.3mm) particles produced the highest yield of oil from SFE process at constant operation pressure. For larger size particles (0.9mm), poor quality yields of turmeric oil resulted even at an elevated temperature. It is because of the increasing number of intact cells that kept a larger quantity of oil inaccessible to the solvent.

The interaction effect of extraction temperatures and particle size on OECs of turmeric oil is shown in Fig.7.36. From the slopes of these graphs it can be observed that SFE of finely milled turmeric samples (DP1) in annulus extraction vessel recovered oil at faster rate and produced higher amount of yields for the operation combinations (T3-DP1 and T1-DP1) compared with largest size particles for same levels of operation parameters (T3-DP3 and T1-DP3). Thus Fig.7.36 shows that the selection of particle size is very much crucial in the SFE of turmeric rhizomes to achieve higher oil recovery.

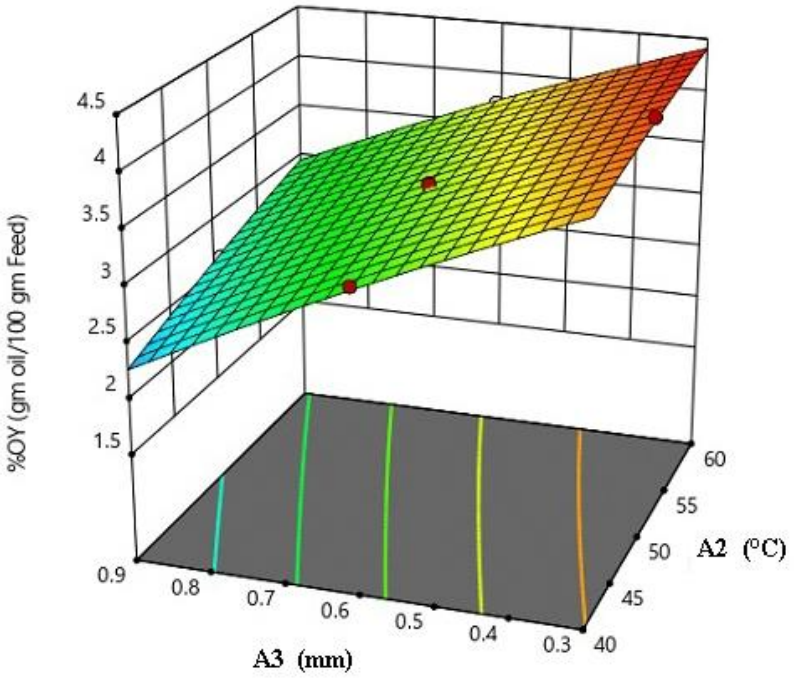


Fig.7.35 : Temperature (A2)– Particle Size (A3) Interaction Effect on Extraction Yield

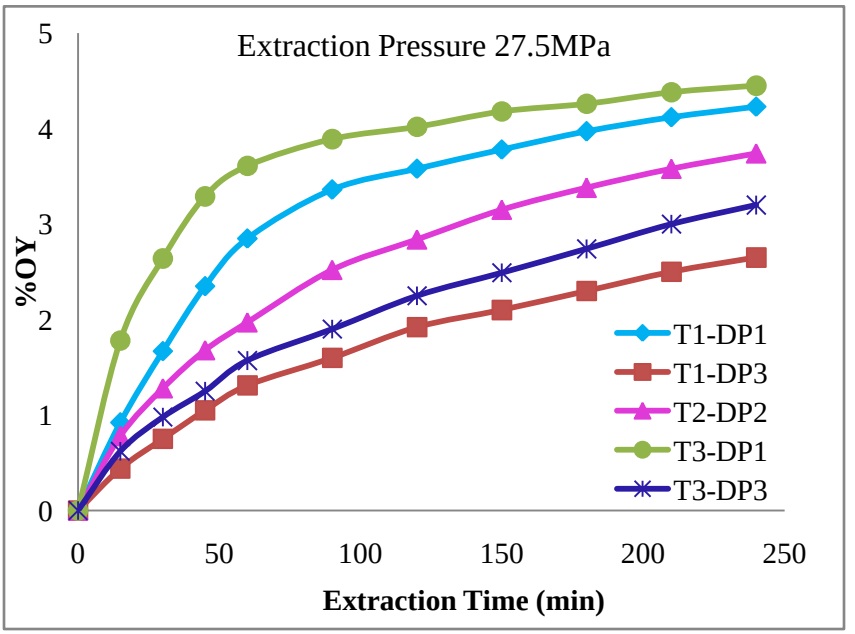


Fig.7.36: Temperature – Particle Size Interaction Effect on Overall Extraction Curves of Turmeric Oil

7.2.3.4 Optimum Conditions of Operating Parameters to Maximize the Yield

The main objective of the present study was maximize the yield of essential oil by optimizing a supercritical carbon dioxide extraction process that extracted turmeric oil from its dried ground rhizomes with introduction of annulus geometry inside the main extraction vessel by incorporating an annulus shell loaded with solid feed into the annular space in place of conventional cylindrical geometry. Analysing the FC-CCD experimental results, Design-Experts numerical optimization was carried out for three chosen operation factors (A1= extraction pressure, A2=extraction temperature, and A3= particle size of ground turmeric rhizomes) to extract maximum yield of turmeric sample. The optimum conditions of the operating parameters found were 27.1MPa extraction pressure, $59.96 \approx 60^0$C extraction temperature, and 0.3mm particle size. The maximum predicted extraction yield ($\%OY_{max}$) of turmeric oil was 4.454%. Supercritical fluid extraction experiments were carried out in triplicate, applying these optimal conditions and same milled turmeric sample to verify the response. The average yield ($\%OY_{exp}$) of these runs was 4.41% that fitted well with the predicted value. The results are provided in Table -7.17.

Table-7.17 : Optimum Values of Operating Parameters to Maximize the Yield of Turmeric Oil

$\%OY_{max}$	$\%OY_{exp}$	Pressure (MPa)	Temperature (°C)	Particle Size (mm)
4.454	4.41	27.1	59.957	0.3

7.2.4 Chemical Analysis of Turmeric Rhizomes Oil

SCO_2E experiments of turmeric rhizomes were performed in the present study to find out bed geometry effect on yield as well as optimize the process to maximize the amount of extract from a constant period of extraction of 240 minutes. Pure solvent SCO_2 without adding any modifier was used for all SFE experiments. Maximum yield of turmeric oil obtained from all these runs was 4.45% that was more than 80% of total oil obtained from Soxhlet extraction. The essential oil content in the dried rhizomes of the plant Curcuma longa usually varied in the range of 3-5% [Gopalan, 2000; Raina, 2002]. The sample of turmeric oil obtained from SFE study is given in Fig.7.37.

This golden spice plant extracts may contain more than 200 bioactive components [Gupta, S. C., 2017]. The main active components present in the turmeric rhizome are aromatic essential oil and curcuminoids. The volatile oil is the contributing factor for the turmeric aroma while the curcuminoids (polyphenol curcumin and it analogous) are the main colouring components responsible for its natural golden yellow colour [Chatterjee, 2000; Dasgupta, 2014]. The volatile aromatic essential oil fractions of turmeric extract were reported to contain mainly different sesquiterpenes (ar-turmerone, α-turmerone, β-turmerone, turmerol), α-atlantone and Curcuminoids (Chang,2006; Govindarajan, 1980; Raina, 2002). The turmeric (C. longa) essential oil of Indian origin was reported to contain 59.7% of ar-turmerone, which is the major

sesquiterpene [Nigam & Ahmad, 1991]. For the presence of active ingredients in a higher percentage (such as turmerone, and curcumin), Indian turmeric oil is considered as the best in quality in the world market.

(Crude Turmeric Oil) (Centrifuged Turmeric Oil)

Fig.7.37: Turmeric Rhizome Oil obtained from Supercritical Carbon-dioxide Extraction

The primary chemical constituents of the turmeric oils obtained in this study from SCO_2E experiments were analyzed using gas chromatograph-mass spectrometry (GCMS) as discussed earlier. The full-length GC-MS chromatogram of turmeric oil is given in Fig.7.38. The peaks in the chromatogram indicated the presence of various bioactive compounds that were fractionated from the turmeric extract. The major identified compounds present in the turmeric oil sample were listed in Table-7.18. Some of the principal components detected were ar-Turmerone(53.51-57.21%), Curlone or β- Turmerone (14.63-17.63%), α -Tumerone (1.21-2.93%), Curcumene 1.49%, α-Atlantone (3.83-5.08%), and ar-Turmerol (2.99-6.32%).

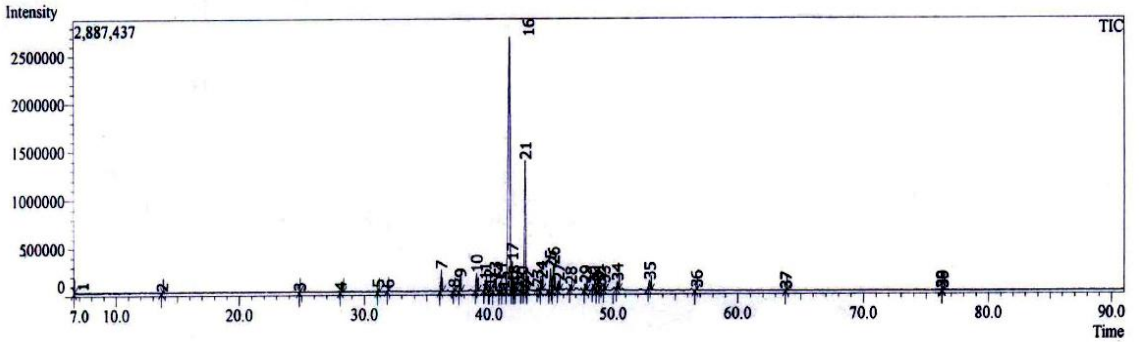


Fig.7.38 : Gas chromatogram of the constituents of essential oil extracted from Turmeric Rhizomes

Table-7.18: Percentage Chemical Composition of the Turmeric Oil

Components Name	Molecular Formula	Molecular Weight	Retention Time (min)	% Con.
Ethanol, 2-Methoxy-, Acetate	$C_5H_{10}O_3$	118.1311	6.551	0.1-0.27
Cymene	$C_{10}H_{14}$	134.222	31.875	0.0-0.16
ar-Turmerol	$C_{15}H_{22}O$	218.34	31.892	2.99-6.32
α - Curcumene	$C_{15}H_{22}$	202.341	36.143	0.0-1.95
ar - Curcumene	$C_{15}H_{22}$	202.341	36.162	0.0-1.49
β-Sesquiphellandrene	$C_{15}H_{24}$	204.357	37.690	0.91-1.26
Lanceol	$C_{15}H_{24}O$	220.356	40.042	0.24-0.28
ar-Turmerone(dihydro)	$C_{15}H_{22}O$	216.324	40.481	1.63-1.77
β-Biotol	$C_{15}H_{24}O$	220.356	40.91	1.14-1.45
Formic Acid, Benzoyl-,(8'-phenylmethyl) ester	$C_8H_8O_2$	136.15	41.250	0.29-0.36
ar- Turmerone	$C_{15}H_{20}O$	216.324	41.68	53.51-57.21
α –Tumerone	$C_{15}H_{22}O$	218.34	41.868	1.21-2.93
1,5-Heptan-4-ol, 3,3,6-Trimethyl	$C_{10}H_{18}O$	154.253	41.995	0.45-0.61
Bisabolol	$C_{15}H_{26}O$	222.372	42.157	0.0-0.11
Carveol	$C_{10}H_{16}O$	152.237	42.633	0.0-0.7
Curlone or β- Turmerone	$C_{15}H_{22}O$	218.34	42.922	14.63-17.63
Curcuphenol	$C_{15}H_{22}O$	218.34	43.150	0.0-0.45
Bisabolone	$C_{15}H_{24}O$	220.356	44.257	1.17-1.31
Pentadecane, 2-methyl-2phenyl-	$C_{22}H_{38}$	302.546	44.939	0.0-2.77
α-Atlantone	$C_{15}H_{22}O$	218.34	45.22	3.83-5.08
α-Tumerone	$C_{15}H_{22}O$	218.3346	46.603	0.43-0.5
Cyclohexane, (2-Nitro-2-Propenyl)	$C_9H_{15}NO_2$	169.2209	47.797	0.73-0.93
γ-Atlantone	$C_{15}H_{22}O$	218.34	48.432	0.50-1.0
Cyclohexanecarboxylic acid, 3-phenylpropyl ester	$C_{16}H_{22}O_2$	246.35	48.787	1.0-1.04
Benzene, (1-cyclopenten-1-ylsulfonyl)	$C_{11}H_{12}O_2S$	208.277	48.975	0.0-0.3
α-Oxobisabolene	$C_{15}H_{24}O$	220.3505	49.317	0.52-0.88
5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	$C_{15}H_{26}O_2$	238.371	50.100	0.0-0.24
2-Methyl-4-octenal	$C_9H_{16}O$	140.226	52.967	1.3-1.47
2,5-Heptadien-4-one,2,6-Dimethyl	$C_9H_{14}O$	138.21	56.759	0.14-0.26

Rest components were present in the range of 0.01 - 0.14

Raina et al. (2005) reported on the chemical composition of the turmeric oil originated from the lower Himalayan region of northern India and extracted by hydrodistillation. The main components of rhizome oil were isolated and quantified were α-turmerone (44.1%), β-turmerone (18.5%) and ar-turmerone (5.4%). The yield of major sesquiterpene, ar-turmerone, was very poor compared to good quality Indian Oil [Nigam & Ahmad, 1991]. Gopalan et al. (2000) obtained 42% ar-Turmerone and 16.12%β-Turmerone from supercritical turmeric extract. Priyanka and Khanam (2018) carried out SCO_2E experiments on turmeric rhizomes and identified 14 bioactive compounds by GC-MS analysis. The principal compounds of turmeric oil were quantified as ar-Turmerone (31-67.1%), β-Turmerone (2-37.9%), and α-Turmerone (0-21.3%). Comparison of the results of chemical analysis of turmeric oil from various literature, it can be demonstrated that the turmeric oils produced from modified annulus extractor bed by SCO_2E were very good in quality since the principal hydrocarbon sesquiterpenes represented more than 70% of the extracted oil.

7.2.5 Dynamic Mathematical Model of OECs

The OECs obtained from different experiments of RSM study were found to fit in the Luo Denglin dynamic model type equation [Luo, D. L., 2013] expressed earlier by equation (7.2)-

$$Y = Y_{\infty}\left[1-\exp(-kt)\right] \qquad \text{----------(7.2)}$$

where Y means the amount of oil extracted at time t. It is expressed as %OY;

$Y_{\propto}$ is a measure of the maximum value of Y after infinite time.

k is a rate constant.

The rate constant, k, was expressed as a function of reduced temperature $\left(\frac{T}{T_c}\right)$ and reduced pressure $\left(\frac{P}{P_c}\right)$.

It was defined as – $$\left[k = A\times\left(\frac{T}{T_c}\times\frac{P}{P_c}\right)\right] \qquad \text{----------(7.3)}$$

where A is proportionality constant;

T_C = Critical temperature of solvent CO_2 = 31.1^0C and

P_C = Critical pressure of solvent CO_2 = 7.39MPa

Substituting 'k' from equation (7.3) in equation (7.2), the kinetic model equation takes the form-

$$Y = Y_{\infty}\times\left[1-\exp\left(-A\times\left(\frac{T}{T_c}\times\frac{P}{P_c}\times t\right)\right)\right] \qquad \text{----------(7.4)}$$

'Y_{∞}' and 'A' are two model parameters. Y_{∞} was substituted from the global yield data obtained from Soxhlet experiment of ground turmeric sample. Another model parameter 'A' was estimated analysing the data concerning the overall extraction curves with the help of software Curve Expert 0.005.

The highest yield of turmeric oil obtained in the Soxhlet process was 5.42%. It was defined as the global yield. Hence, the value of $Y_{\propto}$ was replaced by 5.42. The value of the proportionality

constant 'A' was evaluated as 0.012. The dynamic model equation of the overall kinetic extraction was thus found to take the form:

$$Y = 5.42 \times \left[1 - \exp\left(-0.012 \times \left(\frac{T}{T_c} \times \frac{P}{P_c} \times t\right)\right)\right] \quad \text{----------(7.5)}$$

It described the OECs obtained from SFE of ground turmeric rhizomes quite well (with R^2 ranges from 0.9-0.99). Fig. 7.39(a-c) represents model fitting curves and their corresponding actual OECs of turmeric extract. The operating conditions of extraction pressure and temperature and particle size are provided in the figures. OECs obtained from Optimum bed geometry and a moderate to a high level of operating conditions of pressure and temperature (B3, annulus extractor bed with the larger inner channel), which resulted in the increased of yield, were found to fit well with the predicted model equation.

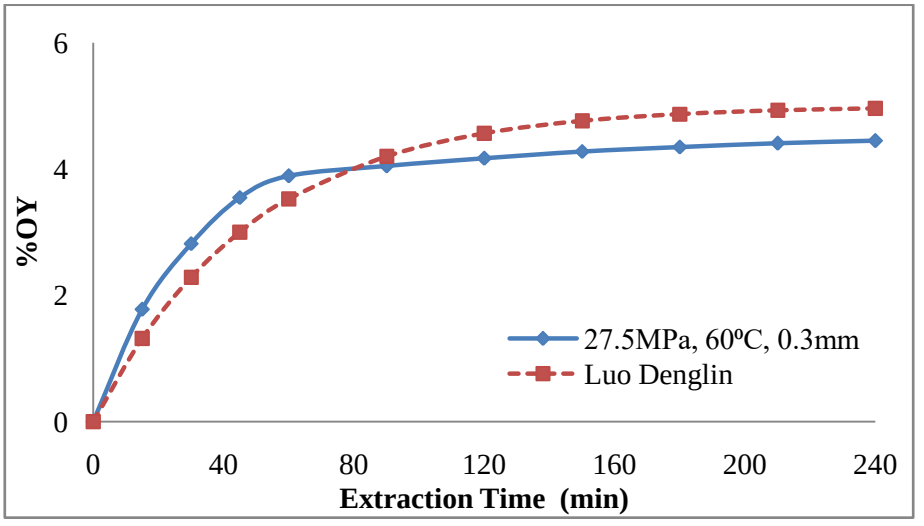


Fig.7.39 (a)

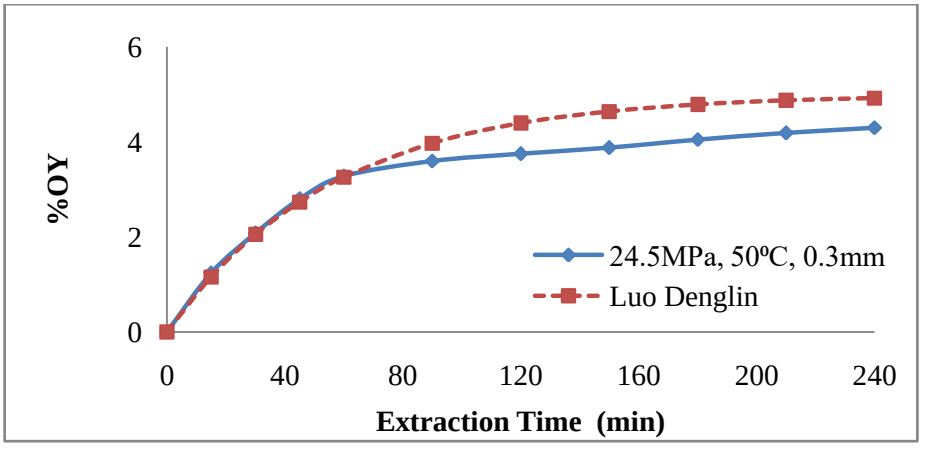


Fig.7.39 (b)

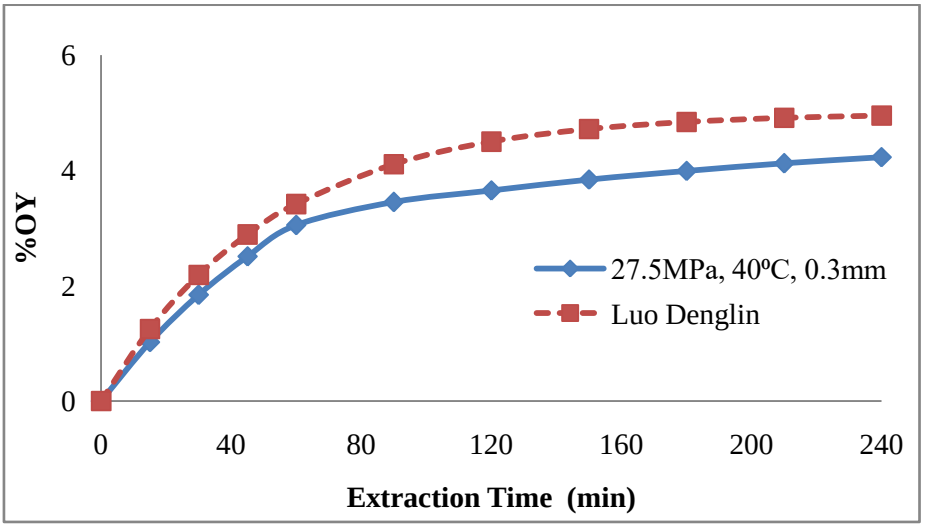


Fig.7.39 (c)

Fig.7.39: Experimental and Predicted Overall Extraction Curves for turmeric Rhizomes under different experimental conditions used in annulus extractor bed B3

7.3 Total Phenol Content and Antioxidant Properties of Supercritical Extracts of Clove Oil

Clove essential oil has been reported to exhibit high antioxidant activity as compared to other plant extracts (Chaieb, 2007; Politeo, 2006). Table-7.19 shows the total phenolic content and antioxidant activities of the Supercritical extracts obtained from Clove buds.

❖ **Total Phenol content (TPC)**

Phenols are crucial plant antioxidants that scavenge free radicals and play a pivotal role in stabilizing lipid oxidation. Previous reports for human indicated that consumption of 1 g of polyphenolic compounds from the diet rich in fruits and vegetables could inhibit the chances of mutagenesis and carcinogenesis (Tanaka 1998). Fig.7.40 shows the standard calibration curve y = 0.0015x + 0.014 of Gallic acid evaluated for the determination of TPC, where x = concentration of standard gallic acid in µg per ml and y = absorbance. Phenol content was estimated to be 852.74 mg GAE/g of clove extract (Table-7.19). The result of TPC of clove essential oil was very much impressive, which revealed that the clove oil extracted using SCO_2 in annulus extractor bed geometry was superior in quality. The result of TPC value evaluated from clove oil was higher than the result reported by Ivanovic (530.56 mg GAE/gm extract) for supercritical clove oil [Ivanovic, 2013].

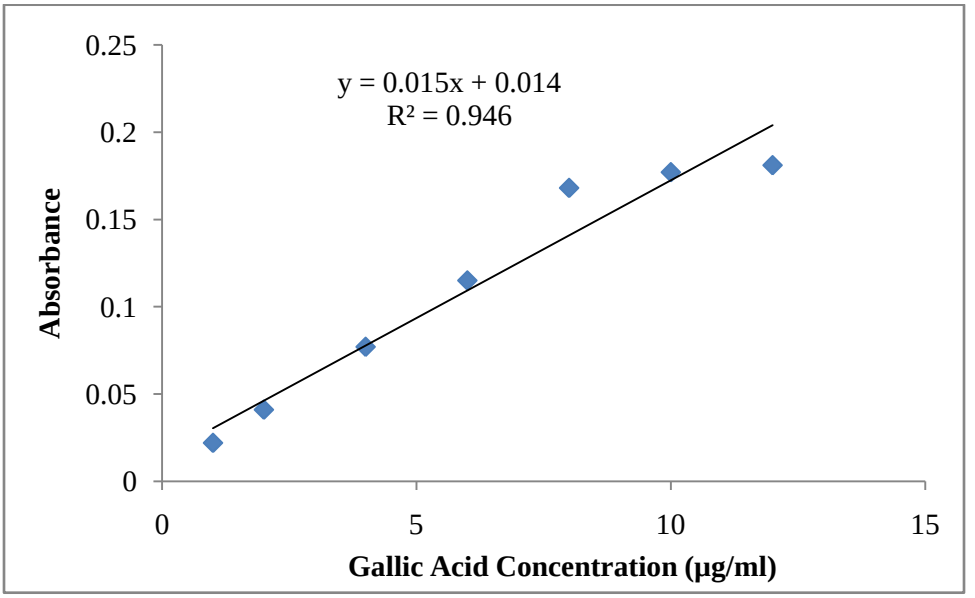


Fig. 7.40: Standard calibration curve of Gallic acid for the determination of TPC

❖ **DPPH• free radical scavenging:**

DPPH$^{\bullet}$ is a stable free radical which accepts an electron or a hydrogen atom to convert itself into a stable diamagnetic molecule. It is a simple and a rapid method to consider how efficiently the antioxidant would react with the free radical and satisfy it by providing it an electron (Yokozawa et al., 1998). Same is measured here for clove essential oil extracted using carbon dioxide solvent by SFE method and compared with standard antioxidant, gallic acid. The EC_{50} value of DPPH$^{\bullet}$ free radical scavenging activity of clove essential oil is 19.13 µg/mL and for gallic acid, the value of EC_{50} is 6 µg/ml (Table-7.19). Graph (Fig. 7.41) was obtained by plotting different concentrations of clove oil against the percent inhibition.

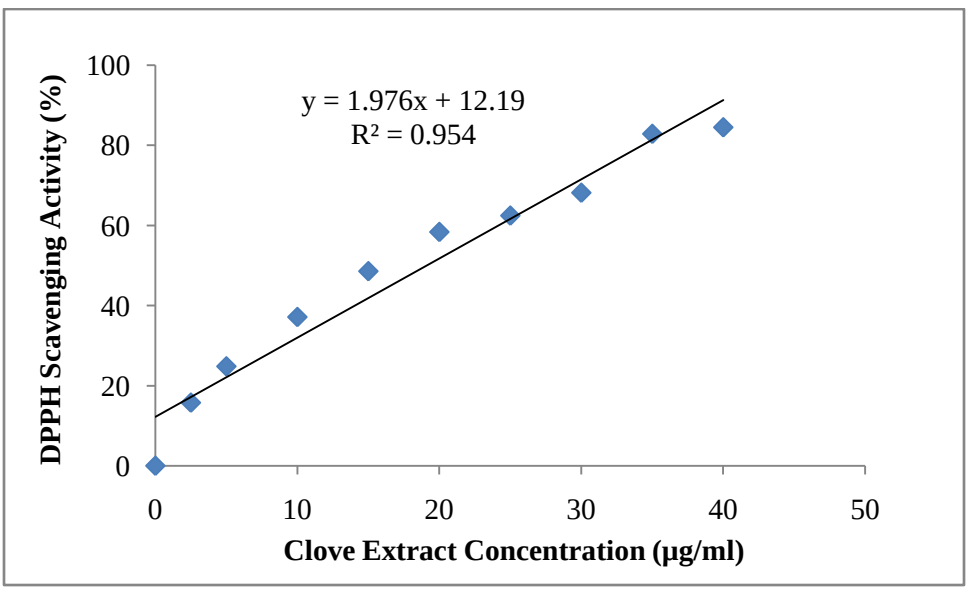


Fig. 7.41: DPPH radical scavenging activity of Clove Oil

❖ **Metal chelating capacity:**

Clove essential oil or any synthetic antioxidant (here BHT) forms a chelate with which like any other transition metal ion is a catalyst in the Fenton reactions. Thus an antioxidant exhibiting metal chelating property reduces multiplication in the number of free radicals due to Fenton reactions. There is, however, a strong correlation between metal chelating and lipid peroxidation or other antioxidant properties. Metal chelating activity by a molecule can reduce ROS production in Fenton reactions. Clove essential oil and BHT was assayed for the metal chelating property. BHT was used for comparison of the result evaluated with clove essential oil.

EC_{50} value for the metal chelating property of clove essential oil was 62.33 ug/ml compared to BHT, which was 42 ug/ml (Table-7.19).

❖ **Reducing Power**

Potential of the antioxidant substances (here clove essential oil and gallic acid) to reduce transition metal ions to their lower oxidation state is an important parameter of study. EC_{50} value for reducing power of clove essential oil was 27 ug/ml and that of gallic acid is 26 ug/ml (Table-7.19).

❖ **Lipid peroxidation inhibition:**

Lipid peroxidation of polyunsaturated fatty acids leads to the formation of malondialdehyde (MDA) as the by-product of the primary and secondary lipid peroxidation. Thio barbituric acid (TBA), which forms the TBARS assay, is used to detect MDA present in the sample. The ferricyanide induced lipid peroxidation in the sample is thus reflected in MDA generation. Percentage MDA generation or the percentage The EC_{50} value for inhibition of lipid peroxidation by clove essential oil and gallic acid are 25 and 28 ug/ml, respectively (Table-7.19). Addition of 100 ug/mL of clove essential oil causes the MDA formation to go down by 70 percent.

Table-7.19:Total phenolic content and antioxidant activities of the Supercritical extracts of Clove

Sample	TPC (mg of GAE/g of Extract	DPPH (EC_{50}) (µg/ml)	FICA (EC_{50}) (µg/ml)	FRAP (EC_{50}) (µg/ml)	LPIA (EC_{50}) (µg/ml)
Clove oil	852.74	19.13	62.33	27.144	25.11
Gallic acid	-	6	-	26.04	28
BHT	-	-	42.33	-	-

Clove essential oil has been reported to exhibit high antioxidant as compared to other plant extracts (Chaieb, 2007; Politeo, 2006). Ivanovica (2013) reported clove oil extracted using SFE

technology has great potential as a natural antioxidant with comparable activity to synthetic antioxidants. The present study clearly indicates that clove essential oil has powerful antioxidant activity against various oxidative systems in vitro. In fact, in all the oxidative systems tested, clove oil has antioxidant activity comparable to that of gallic acid and BHT. Therefore, clove essential oil may be used as a source of natural antioxidants and possible food supplement, with potential application in the pharmaceutical industry.

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Chapter 8

CONCLUSION AND SCOPE OF FUTURE WORK

8.1. Conclusion

The operating parameters as well as conditions for maximum global yield in the extraction with supercritical carbon dioxide of clove bud oil and turmeric rhizome oil were predicted for the developed extractor using FC-CCD technique. Effects of two main operating parameters-temperature and pressure on extraction yield at three different bed geometry (expressed as axial to radial surface enhancement factor) and particle size were investigated and evaluated. Conditions of oil bearing substrates were also found to play a major role in extraction process in terms of both the quality and quantity of the products. RSM methodology of statistical analysis was used to analyze effects of interaction of two different process parameters and the optimum condition of the SFE process. All the data were correlated by a mathematical expression to predict the oil yield within the range of experimental conditions of operation applying ANOVA. Dynamics of the extraction curves were also established separately by fitting in the Luo Denglin dynamic model type equation.

The procedures, which were developed for this study, are sufficiently precise to indicate that the products are superior in quality. Highest eugenol content of 72.08% was achieved from the extract of clove obtained from optimal condition along with other main components of eugenyl acetate 11.84% and caryophyllene 6.73%. Similarly, ar-Turmerone content in the range 53.51-57.21% was achieved from turmeric extract, which is also indicated a good quality yield as highest value was reported as 59.7% for best quality Indian turmeric oil. Presence of a larger percentage of main compounds was noticed using annulus extractor bed geometry as compared with other conventional studies.

Different antioxidant assays such as total phenolic content (TPC) using folin-ciocalteu reagent (FCR), free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), metal chelating capacity (Fe^{2+} ions chelating assay), potassium ferricyanide reducing power, and lipid peroxidation inhibition of extracted oils were determined and found to be excellent.

Some of the important inferences of the research work are:-

- Studies on various operating parameters, temperature, pressure, particle size and bed geometry, indicate that the increasing temperature and pressure in the range of experimental design have a positive impact on the rate of extraction and hence in reduction of extraction time.
- Annulus bed geometry shows significant enhancement on the rate of extraction and %OY over conventional cylindrical geometry under same operating conditions for extraction of both plant materials - clove buds and turmeric rhizomes.
- Smaller size particles makes the extraction faster, it plays a major role to extract oils close to the maximum global yield.
- Chemical analysis of extract obtained from clove buds and turmeric rhizomes using SFE method and annulus bed geometry revealed that introduction of annular channel inside the cylindrical extractor may improve the quality of the product.

- For clove bud oil extraction, ARSEF value of 0.0238 for annulus extraction vessel gave optimum oil yield at an extraction temperature of 44.72°C and an extraction pressure of 24.5MPa. The maximum predicted extraction yield of clove oil was 17.981%.
- For turmeric oil extraction, the optimum conditions of the operating parameters were found to be 27.1MPa extraction pressure, 60^0C extraction temperature and 0.3mm particle size. The maximum predicted extraction yield of turmeric oil was 4.454%.
- The effect of axial to radial surface area enhancement factor, $[(r_o-r_i)/2L]$, seems significant as extraction time reduces and oil yields increase with the decrease of this factor. But after certain values of the enhancement factor, this became insignificant. This may be due to the fact that the channeling effect became pronounced at smaller values of this enhancement factor.

8.2. Future Scope of Work

Since CO_2 is a potent green house gas, more uses of this gas in circulatory system will tantamount to sequestration. So, more applications of SCO_2E in circulatory system should be encouraged and hence further work is required to get full access of the developed experimental procedures in non-venting systems particularly during charging and discharging of feedstock.

Though decrease in axial to radial surface enhancement factor has a positive effect on SCO_2E, its benefits after an optimal value gradually become flattened due to pronounce channeling effect. It is not clear at this stage, at least, how hydrodynamic behavior of the feed bed influences the extraction curve. Further systematic studies with different bed geometries in order to correlate hydrodynamic behavior are necessary for establishing a suitable criterion that can be used to predict extractor performance along with its economic gain.

More investigations need to be done to scale up the annulus extractor bed and optimize the annulus bed geometry to operate successfully without channeling effects that may lead to poor performance of the extractor.

It is noted that the quality of compounds obtained by SCO_2E from the same matrix is very superior in quality. However, the yield of the extraction process is often lower, which could indicate a higher selectivity in the extraction of bioactive components. SCO_2E offers a more selective and environmentally sustainable alternative to traditional methods in natural products extraction and do not require organic solvents. But as evident from the observations of the effects of bed geometry on extraction, mass transfer plays an important role in it. So, the use of ultrasonic-assisted supercritical fluid extraction may offer greater improvements in the yield of bioactive compounds due to a high mass transfer rate and require further investigation.

ANNEXURE - 1

COPY OF PUBLISHED PAPERS

Experimental Investigation on efficient Supercritical CO_2 Extraction of Essential Oil from Turmeric Rhizomes: Effects of Geometric and other Operation Parameters

Sutapa Roy, Chandan Guha, Asit Kumar Saha, Somak Jyoti Sahu

Abstract*: Supercritical carbon dioxide extraction experiments were carried out to isolate essential oil from turmeric rhizomes efficiently using extractors of annulus bed geometry and conventional cylindrical geometry using the same operating conditions of pressure 24.5 MPa, temperature 50^0C and particle size 0.3 mm keeping the time of extraction constant. A faster rate of extraction and improved yield was obtained in annulus bed geometry than conventional cylindrical geometry. The effect of pressure, temperature and particle size within the range of 21.6 MPa to 27.5 MPa, 40^0C to 60^0C and 0.3 mm to 0.9 mm respectively in annulus bed geometry were studied using response surface methodology. Full face central composite design method of statistical analysis was applied to find the interactions of all these parameters on oil yields and the optimum conditions. It was found that optimum oil yields of 4.454 gm oil/100 gm turmeric powder were obtained at a temperature of 59.96^{0}C and a pressure of 27.097 MPa for an average particle size of 0.3 mm. Model equations predicting the oil yields with operating parameters were also proposed.

Keywords: Supercritical carbon dioxide extraction, turmeric oil, extractor bed performance, oil yield, central composite design.

I. INTRODUCTION

Turmeric rhizomes are finger-like underground storage organs obtained from a perennial, tuberous herb (*Curcuma longa L.*) belongs to the Zingiberaceae family [1]. Its uses in traditional Chinese medicine and ayurvedic medicine of India were reported as more than thousands of years older [2]. Among different warmer parts of the world where turmeric is cultivated extensively, India is one of the largest producers of commercial turmeric products like turmeric powder, essential oil, oleoresin, etc. [3] and exporter as well [4]. The dark yellow powder product processed from dried matured rhizomes is used as a daily spice by almost one billion populations over the world for its natural color pigments, flavor, aroma, and food preservation characteristics [5]. There are several amazing benefits of daily use of raw turmeric rhizomes. Major industries who consume turmeric in many ways include foods, pharmaceuticals, confectionery, cosmetics and textiles [4, 6].

This golden spice plant extracts may contain more than 200 bioactive components [7]. These active ingredients of turmeric consist of mainly essential oil (volatile aromatic fractions) and nonvolatile saffron color polyphenol curcumin (probably the strongest antioxidant of turmeric) [5]. The volatile oil of Curcuma from Indian origin was reported to contain mainly four different sesquiterpenes (ar-turmerone, α-turmerone, turmerol, and β-turmerone) [8]. The benefits of these secondary metabolites were enlisted as having anti-oxidative, anti-inflammatory, antifungal, antibacterial, anti-carcinogenic, anti-mutagenic, antiviral, insect repellant, anticoagulant, ant diabetic, antiprotozoal, antivenom, antiulcer, antifibrotic, antifertility, hypotensive and hypocholesteremic properties [9, 6].

The extracts from various parts of *C. longa* are possible to recover by various extraction methods such as Soxhlet extraction [10-11], steam distillation [12] , hydro-distillation [1, 8], microwave-assisted extraction [13] and supercritical fluid extraction (SFE) using supercritical carbon dioxide (SCO_2) [14-16, 6]. Among them, two mostly used laboratory and industrial grade traditional methods, hydro-distillation, and steam distillation, are suffering from producing good quality yields [16]. Supercritical carbon dioxide extraction (SCO_2E) is a robust technology to produce good quality yield with abundant bioactive components [15], provide oxygen-free extraction environment, minimize extraction time and solvent consumption, reduce secondary treatment steps, and diminish solvent contamination of the product to zero levels [14]. Various research works were reported to study the effect of various operating parameters such as pressure, temperature, particle size, solvent flow rate, the addition of co-solvent, and material drying conditions on the extraction of turmeric oil from different plant parts using SFE. Gopalan, Priyanka, and their co-workers recommended a pressure range from 20MPa to 40MPa and temperature range from 313K to 333K for SCO_2E of turmeric rhizomes to obtain good quantity oil yield [14, 6].

Revised Manuscript Received on June 05, 2019

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Chang and others suggested an operating pressure of at least 26MPa and the temperature range of 320K-350K is suitable for turmeric oil extraction using SCO_2E without applying any co-solvent [15].

Some studies on the effect of particle size indicate that particles smaller than 0.45 mm enhance the rate and yield of extraction using SCO_2 [6, 15]. Angel L. Chassagnez-Me´ndez studied the kinetics of SCO_2E of curcumins and essential oil from the turmeric rhizomes [17]. Another important factor that has considerable influence on the extraction process along with various operating conditions of SFE is the design of extractor. The effects of design variations of the extractor (in terms of bed volume, bed height to diameter ratio, etc.) on SFE of various plant materials were reported in some works of literature [18-24]. The variation in bed geometry interferes in the extractor performance by affecting the distribution pattern of solid feed, the tortuous path for solvent flow, mass and heat transfer rates. These factors influence the overall extraction curves (OEC) and corresponding extraction kinetics. Conventionally cylindrical geometry is used in designing the extractor vessel. In the present work, a special type of annular extractor bed design, introduced earlier by us to study the effect of geometrical modification of extractor on supercritical carbon dioxide extraction of clove bud oil [25], is used for turmeric oil extraction. It aims mainly to establish the effect of this design concept in the extraction of biomass like turmeric rhizomes which is not enriched with essential oils like clove buds. The effects of operating parameters like pressure, temperature and particle size using modified bed geometry are also studied. Then the performance of modified extractor beds in the extraction of turmeric oil was compared and verified with the results obtained earlier in the case of clove oil extraction.

II. MATERIALS AND METHOD

A. Plant Materials and Chemicals

The quality matured dried turmeric rhizomes as available in local market were obtained from Haldia (West Bengal, India), checked minutely to remove any other impurity (if any) and dried under controlled condition in a laboratory drying unit having air evacuated system for 12 h. This is done in order to avoid the opposing effect of moisture content of the sample above 12% on the rate of mass transfer and solubility of the volatile matter in the solvent CO_2 used for SFE [26].

The dried turmeric sample was then milled in a mixture grinder (Philips Mixer Grinder HL7720) and separated into three fractions with the help of a sieve shaker assembled with 16 - 80 mesh sieves from Tyler standard screen series. The average particle sizes (D_P) of different fractions were determined following the mass mean diameter calculation and the ground turmeric (to be used later for SCO_2E experiments) was stored after packaging in air-tight polyethylene bags in cold and dark place since curcuminoids, the natural pigment of turmeric, degrade in contact with light, heat and oxidative conditions [27].

CO_2 (commercial grade with above 99% purity) used in the extraction experiments was acquired from a local supplier, Bharat Oxytech Pvt. Ltd., Haldia (West Bengal, India).

B. Moisture Content of Turmeric Rhizomes

The moisture content of both, the raw turmeric bought from the market and moisture controlled turmeric after laboratory drying, were measured using the "SARTORIUS MA45C" moisture analyzer. The moisture analysis results were provided after triplicate measurements.

C. Determination of the Global Yield (Total Amount of Extractable Material)

In the present study, the traditional Soxhlet extraction method was applied to obtain the entire extractable aromatic oil content from the turmeric sample. For the experiment 30 gm of the dried and comminuted sample from the feedstock having 0.6mm average particle size was loaded in a glass thimble after wrapping in Whatman filter paper. The thimble was connected with a round bottom reflux flux (500 mL capacity) of the Soxhlet apparatus. The extraction was carried out using 200 mL n-hexane as a solvent and the apparatus was kept under reflux condition for 8hrs. Then the final extract was separated by removing the solvent at 50^0C with the help of a rotary vacuum evaporator.

D. Modified Externally Loaded Solid Bed

It is common practice to fill the ground plant material externally in a shell (conventionally cylindrical in geometry) having perforated surface and placed it inside the cylindrical extractor vessel for solid-fluid SCO_2E. In this study, a concentric tube extractor shell was used to study the effect of this modified bed geometry on turmeric oil extraction as a continuation of our previous published work [25]. This special type of extractor shell is an assembly of two concentric perforated tubes. The smaller diameter tube which has one blind end at the upstream side was surrounded by the main shell of fixed diameter 5.5cm (same as the internal diameter of the extractor vessel) and groundmass of plant matrices was loaded in the annulus. The internal tube was designed for two different diameters (0.75cm and 1.5cm). Experimental studies on turmeric oil extraction using supercritical carbon dioxide (SCO_2) were conducted under same operating conditions using modified extractor bed of two different dimensions (AB1, Annulus Bed designed with 1.5cm diameter inner channel & AB2, Annulus Bed designed with 0.75cm diameter inner channel) and conventional solid bed without any annulus (CB) to co-relate the bed performances with previous study using same alteration of bed geometry [25]. The figure of annulus bed loaded inside the extractor was available in the previous publication [25].

E. SFE Experimental Set-up

All the experimental investigations related with the evaluation of the annulus extractor bed performance in turmeric oil extraction by SFE were conducted using a semi-batch type SFE unit (Model No: CSL/SCF/1L2/400) supplied by M/s Chemtron Science Laboratories Pvt. Ltd. (Navi Mumbai, India), and described elsewhere [25].

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It consists mainly of a high-pressure pump, a SCO_2 generation vessel, two 1000mL Extractors (each of 42cm height and 5.5cm inside diameter), and two low-pressure 1000mL Separators, and a low-temperature CO_2 Storage and a Control Unit to view and change the system settings. The schematic of the SFE module used in the present study is the same as published earlier [25].

F. Operational Procedure

The operational procedure used in this SFE module was described in detail by S.Roy et al [25]. Initially, a particular type of feed shell (designated as AB1, AB2 or CB) was filled in full with comminuted turmeric samples and placed inside the extractor vessel to carry out the runs. Pressurized solvent CO_2 from the pump was allowed to enter the extractor vessel through SCO_2 generation vessel to attain the desired extraction pressure. Once the extractor pressure was stabilized, the extract laden SCO_2 was expanded to reduce the pressure and recover the essential oil through two successive separators. In all the experiments extraction was continued for a period (t_E) of 240 minutes and the samples were collected and weighed at intervals (of 15, 30, 45, 60, 90, 120, 150, 180, 210, & 240 minutes) using separate sampling bottles and recorded to construct OECs. This SFE unit was equipped with a solvent CO_2 recovery system and the recovered solvent was returned back to the low-temperature CO_2 storage vessel for reuse. After extraction total yield was centrifuged and the pure essential oil part was separated and stored in a refrigeration unit for further analysis.

G. SCO_2E using Different Bed Geometry

SFE runs to isolate essential oil from turmeric rhizomes using extractors of annulus bed geometry (AB1 & AB2) and conventional cylindrical geometry (CB) was conducted to see the impact of annulus bed geometry over conventional cylindrical geometry and compared with the results as obtained in case of clove oil extraction using SCO_2 in the previous study [25]. Extraction experiments of milled turmeric powder of the same particle size were carried out in the same experimental setup, applying the same operating conditions and the same period of extraction, varying only the bed geometry of extractor vessel. All the experimental data such as mass of feed (F), extraction pressure (P), extraction temperature (T), particle size (D_P), solvent flow rate (Q_{CO2}), initial static period of extraction (t_S), period of extraction (t_E), yield of extract (as %OY) are provided in Table-1. The extract of oil was expressed as percentage oil yield [%OY = (gm of oil extract /100 gm of extractable mass]. All the assays were replicated twice for double sanguine. Finally, the OECs for all these three-bed geometries were plotted and compared.

Table-1: Experimental Data from the Extractor Performance Study

F (gm)	D_P (mm)	Q_{CO2} gm/min	T in E (^{0}C)	P in E (MPa)	T in S_1 (^{0}C)	P in S_1 (MPa)	T in S_2 (^{0}C)	P in S_2 (MPa)	t_s (min)	t_E (min)	Q_{CO2} gm/min
500	0.3	10	50	24.5	30	≈6	25	≈5	20	240	18.5

F - Mass of feed, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, E – Extractor, S_I – Separator-I, S_{II} – Separator-II, t_s - Static period, t_E period of extraction

H. Experimental Design and Statistical Analysis

The performance of an extraction process to produce essential oil from various plant parts is evaluated in terms of the quantity of extract obtained from the process and the quality of the yield measured in terms of the presence of important bioactive components in dense form. The quantity and quality of oil extracted from vegetable matrix by SFE are influenced by various operating parameters, such as temperature, pressure, particle size, solvent flow rate, time of extraction, use of co-solvent, level of moisture in the feed, porosity of feed bed, extractor bed geometry [28], and their roles on the process may be direct/indirect also independent/interactive in nature [29]. In the present work, the extractor with high performance as obtained from the experimental results of Section-G was chosen for the parametric study of SFE process on turmeric. Three parameters, (i) pressure (X1), (ii) temperature (X2), and (iii) particle size (X3) were chosen to analyze their role in producing turmeric oil efficiently and optimize them to maximize the yield.

For SCO_2E processes, the statistical optimization procedures were applied extensively to find out optimal operating conditions that ensure either the maximum oil yield or yield with the maximum targeted bioactive component. The methods of statistical analysis examine various possible interactions of the process variables during optimization [30]. In statistics, central composite design (CCD) is a useful tool under Response Surface Methodology (RSM) for modeling various technological processes by fitting a second-order mathematical relation between the process variables and one or more response variable(s). In this work, face-centered central composite design (FC-CCD) strategy was applied to build a statistical model equation that explores the relations between optimizing parameters X1, X2 and X3 of SCO_2E (for a particular bed geometry) and dependent response %OY of turmeric. For experimental design, values of three process parameters X1, X2, and X3 were expressed at three levels as (−1), (0) and (+1) and FC-CCD required responses (%OY) resulting from the experiments conducted for twenty different combinations of these three independent process variables. Three levels of the process variables pressure, temperature and particle size of the present study are given in Table-2. The temperature levels were chosen following the previously published works [6, 15]. In selecting pressure levels, (i) highest pressure of 27.5MPa was chosen considering the design pressure of the extractor (29.42 MPa) and (ii) lowest pressure of 21.6 MPa was selected considering the favorable pressure data recommended for turmeric extraction (at least 26MPa) [15]. Two particle sizes were selected above the recommended size (≈0.45mm) of previous researchers [6, 15] to study the influence of modified bed geometry to overcome the negative impact of larger particle size.

All the 20 experiments of FC-CCD generated combinations were

performed and the analysis of variance (ANOVA) was done using the Design Expert-11 software package [31]. Thus, the influence of each independent factor and their interactions were examined and estimated statistically. All the runs were performed fixing other parameters such as mass of feed (F), solvent flow rate (Q_{CO2}) and period of extraction time (t_E) same as provided in Table-1.

Table-2: Three levels of selected variables chosen for FC-CCD

Pressure, X1 (MPa)	Temperature, X2 (^{0}C)	Particle size, X3 (mm)
21.6 (-1)	40 (-1)	0.3 (-1)
24.5 (0)	50 (0)	0.6 (0)
27.5 (+1)	60(+1)	0.9 (+1)

I. Characterization of Turmeric Extract: GC/MS analysis

The compositions of volatile substances present in the turmeric extract were identified in an advanced standard gas chromatograph *mass* spectrometer, *GCMS*-QP2010 SE (SHIMADZU, Kyoto, Japan). The capillary column, DB - 1 MS UI with specification length 60m, inside diameter 0.25m, internal film width 0.25µm, used for separating the components was supplied by Agilent. The sample of essential oil of turmeric rhizomes was diluted using acetone in 1:4 ratios and injected with the help of an auto injector. 1 µL volume of diluted sample was injected in the split mode (1:50). The other details are given elsewhere [25]. The total run time was 90 min.

The settings of MS detector used in the analysis of turmeric oil were – (i) Ion source temperature 220^{0}C, (ii) interface temperature 300^{0}C. The mass spectra developed by the detector were analyzed to identify the chemical species using GCMS solution software (version 4) build with MS library - NIST, Wiley, and SHIM. All the testing of turmeric samples was done in quality control laboratory of M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

III. RESULTS AND DISCUSSIONS

A. Moisture and Global yield

In Table-3, the moisture content of raw and dried milled samples of turmeric rhizomes and the global yield resulted from Soxhlet extraction experiments are provided. The result of the global yield was found to agree well with literature values [32, 6].

Table-3: Moisture Content and Global yield

The moisture content of raw milled Turmeric (wt %)	The moisture content of dry milled Turmeric (wt %)	Global Yield (gm oil / 100 gm feed)
12.34	4.29	5.42

B. *Effect of Bed Geometry Modification on Extractor Performance*

Fig 1 shows that the bed geometry modification from conventional cylindrical type (CB) to annulus type (AB1 & AB2) influenced the OECs significantly in terms of the rate and yield of extraction while other conditions including extraction time (t_E) were kept constant. The yield (%OY) was maximum in the case of AB1 type extractor and lowest in the case of CB type extractor for a fixed extraction period of 240min. Since operational cost is increased with increasing period of extraction (t_E) to recover at least 90% of the volatile fraction of plant material, the design concept of any extractor that may increase the rate of extraction during constant extraction rate (CER) period in OEC (indicated by the initial steeper portion of the curves) must play a positive role on the process. In the present study on turmeric oil extraction by SCO_2, annulus bed arrangement (AB) revealed that this geometrical modification had some positive impact on the performance of the extractor as reported in the case of clove oil extraction [25]. It is due to the fact that the annulus bed reduces the molecular diffusive path for all the molecules and also induces turbulence in the supercritical fluid bulk that increases convective diffusion. Thus, the mass and heat transfer resistance for oil extraction decreases yielding higher oil mass [25]. During the design of the annulus bed, the detrimental effect of larger voids on channeling must come under consideration.

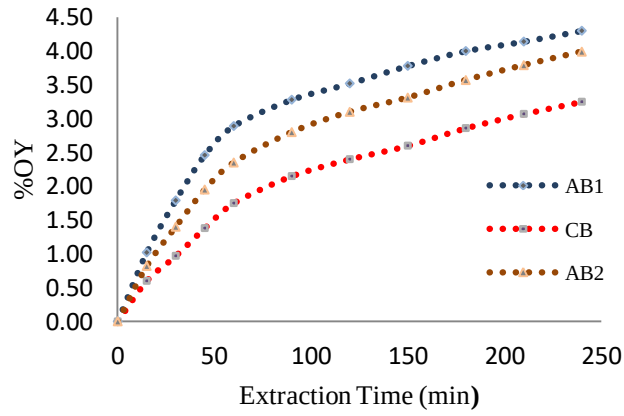

Fig 1: Variation in OECs of turmeric extract under varying bed geometry AB1, AB2, and CB (Process Parameters: Temperature (50^{0}C), Pressure (24.5 MPa), Particle Size (D_p = 0.3 mm)

C. Analysis of Variance (ANOVA)

In this work, RSM was applied to find out the quadratic model equation to optimize three process variables (X1, X2, and X3) and maximize extract [expressed as (%OY)] of turmeric oil extraction process using annulus bed extractor AB1 and SCO_2. Based on the FC-CCD for three factors, a total of 20 experiments of turmeric oil extraction were performed with 20 sets of values of X1, X2, X3 (three factors of CCD). The yield, %OY, obtained from each experiment was reported as the response of CCD along with corresponding values of X1, X2, and X3 in Table- 4.

Table -4: FC-CCD data of three factors and response oil Yield (%OY) of Turmeric rhizome

	Factor 1	Factor 2	Factor 3	Response 1
Run	A:Pressure (MPa)	B:Temperature (^{0}C)	C:Particle Size (mm)	%OY (gm oil/100 gm Feed)
1	24.5 (0)	40(-1)	0.6(0)	3.25
2	21.6 (-1)	40(-1)	0.3(-1)	3.76
3	24.5 (0)	50(0)	0.6(0)	3.51
4	24.5 (0)	50(0)	0.6(0)	3.5
5	27.5 (+1)	50(0)	0.6(0)	3.74
6	24.5 (0)	50(0)	0.6(0)	3.5
7	24.5 (0)	50(0)	0.6(0)	3.52
8	21.6 (-1)	60(+1)	0.3(-1)	4.3
9	27.5 (+1)	60(+1)	0.9(+1)	3.2
10	21.6 (-1)	60(+1)	0.9(+1)	2.7
11	27.5 (+1)	60(+1)	0.3(-1)	4.45
12	24.5 (0)	50(0)	0.6(0)	3.5
13	27.5 (+1)	40(-1)	0.9(+1)	2.65
14	27.5 (+1)	40(-1)	0.3(-1)	4.23
15	24.5 (0)	50(0)	0.3(-1)	4.3
16	21.6 (-1)	50(0)	0.6(0)	3.23
17	24.5 (0)	50(0)	0.9(+1)	2.62
18	24.5 (0)	60(+1)	0.6(0)	3.72
19	21.6 (-1)	40(-1)	0.9(+1)	1.84
20	24.5 (0)	50(0)	0.6(0)	3.5

To explore the model equation, all convenient models such as linear, two-factor interaction (FI) and quadratic were examined for the responses %OY of all runs based on R^2 [33], standard deviation, adjusted R^2, predicted R^2, "PRESS" values, F-values, p-values, and lack-of-fit tests results. The higher order quadratic model was chosen as best for the data from the fit summary [larger F-value (25.69), negligible p-value <0.0001, low value of standard deviation (0.0256), high value of R^2 (0.9992) , lowest "PRESS" value and larger Adjusted R^2 (0.9984) and largest Predicted R^2 (0.994), and (Adjusted R^2 - Predicted R^2) < 0.2]. ANOVA test results as illustrated in Table-5 provided the information about significant fitting of all linear terms (X1, X2, X3), all FI terms (X1X2, X1X3, X2X3) and all quadratic terms ($X1^2$, $X2^2$,$X3^2$) from their individual P value (most of them are less than 0.0500). The Model F-value of 1348.94 implies that the quadratic model equation is significant. The larger value of adequate precision (144.879>>4) obtained in ANOVA was the desirable condition to describe the true behavior of the system by the selected model in comparison to the linear model and 2FI models.

Table 5: ANOVA for the turmeric yield (% OY) in the FC-CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.95	9	0.8836	1348.94	< 0.0001	significant
A-Pressure (X1)	0.5954	1	0.5954	908.85	< 0.0001	
B-Temperature (X2)	0.697	1	0.697	1063.95	< 0.0001	
C-Particle Size (X3)	6.45	1	6.45	9843.39	< 0.0001	
AB	0.0496	1	0.0496	75.74	< 0.0001	
AC	0.0595	1	0.0595	90.85	< 0.0001	
BC	0.0528	1	0.0528	80.62	< 0.0001	
A^2	0.0026	1	0.0026	3.89	0.0767	
B^2	0.0026	1	0.0026	3.89	0.0767	
C^2	0.0085	1	0.0085	12.91	0.0049	
Residual	0.0066	10	0.0007			
Lack of Fit	0.0062	5	0.0012	17.72	0.0034	significant
Pure Error	0.0003	5	0.0001			
Cor Total	7.96	19				

Std. Dev.	0.0256					
R^2	0.9992					
Adjusted R^2	0.9984					
Predicted R^2	0.994					
Adeq Precision	144.8787					

X1, X2, and X3 relates the effects of main process parameters pressure (MPa), temperature (°C), and particle size (mm) on the response (%OY). $X1^2$, $X2^2$, and $X3^2$ produces the quadratic effects of the same input variables. X1X2, X1X3, and X2X3 express the interaction effects of three possible combinations of three factors (i) pressure and temperature; (ii) pressure and particle size, and (iii) temperature and particle size, respectively

D. Model Equation obtained from RSM

The quadratic mathematical model expression representing the percentage oil yield (% OY) of turmeric rhizomes as a function of the three independent process variables of RSM study in the range of their values under investigation is given by the following generalized equation:

$$\%OY = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X3 + \beta_{12} X1X2 + \beta_{13} X1X3 + \beta_{23} X2X3 + \beta_{11} X1^2 + \beta_{22} X2^2 + \beta_{33} X3^2$$

where %OY is the actual response; β_0 is the regression coefficient of intercept; β_1, β_2, and β_3 are the regression coefficients for linear fit; β_{12}, β_{13} and β_{23} are the regression coefficients for FI fit; and β_{11}, β_{22} and β_{33} are the regression coefficients for quadratic fit. The actual values of the regression coefficients of the final regression model were given in Table-6.

Table 6: Coefficient Table of ANOVA Test for turmeric

	β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{11}^2	β_{22}^2	β_{33}^2
%OY	3.50918	0.244	0.264	-0.803	-0.07875	0.08625	0.08125	-0.03045	-0.03045	-0.05545

E. Pressure, Temperature, and Particle size: Effects on the Oil Yield

Influence of individual process parameter on turmeric extract as (%OY) can be described with the help of perturbation plot shown in Fig. 2. The response surface plots are shown in Fig. 3 (a) -(c) were used to explain the two-factor interaction effects on the extract of turmeric in the range of values chosen for investigation. Analysis of both type plots indicates that pressure (in the range of 21.6-27.5 MPa) and temperature (in the range from 40^0C - 60^0C) both show a slightly positive impact on improving %OY. On the other side, particle size shows a significant effect on the extract. %OY was increased notably with decreasing the particle size from 0.9mm to 0.3 mm. Thus combined effect of pressure-temperature on %OY is lower than pressure – particle size interaction or temperature – particle size interaction. The increase of yield with increasing pressure is due to the increase of solubility of the solute with increasing pressure. Similarly, increasing temperature influences the yield positively due to the faster rate of mass transfer of solute attained from high diffusivity and vapor pressure value [28, 34-35].

The reduction of particle size to an optimal level is beneficial as milling of plant material to smaller particle size helps to rupture the cell walls and thus more molecules of volatile oil expose to the surface and come in direct contact with the solvent and easily extracted. In some literature, a range of particle size 0.2-0.45 mm was mentioned where yield increases gradually after which it declines significantly [6, 15]. Thus, a gradual reduction of yield for 0.6mm to 1.0mm particle size in the present study satisfies the literature. In this work RSM was applied in modified annulus bed geometry AB1and extraction was carried out without applying any co-solvent. In terms of percentage recovery of extractable oil at a faster rate annulus bed AB1 performance is remarkable as compared with CB performance under the same operating condition.

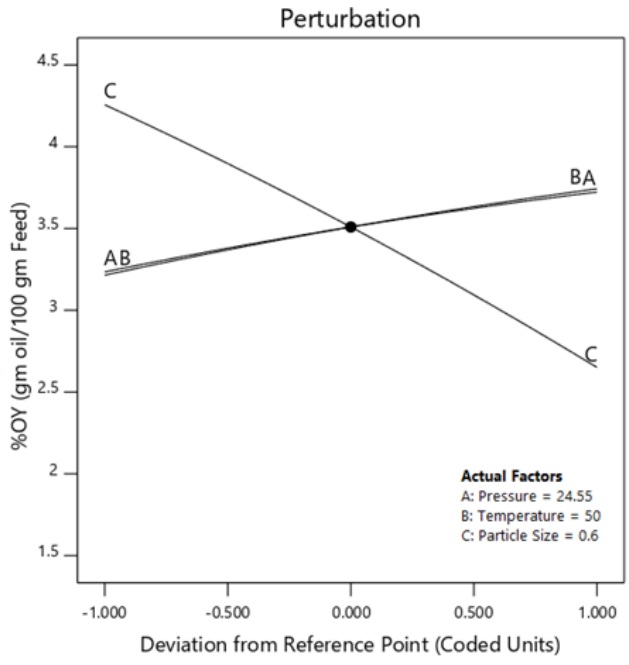

Fig 2: Effect of Individual Process Parameters on the %OY

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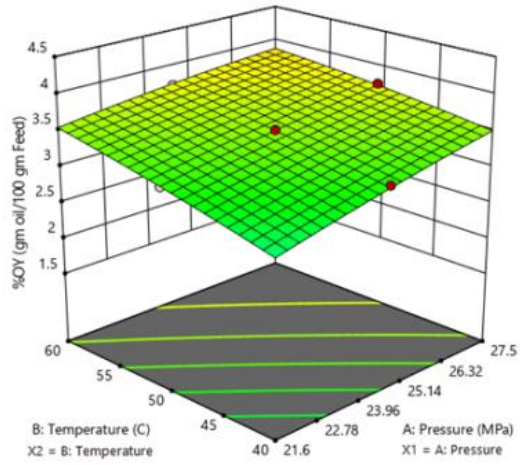

Fig. 3(a)

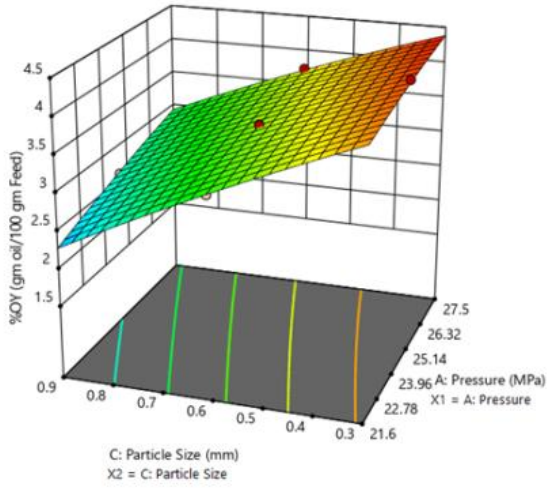

Fig. 3(b)

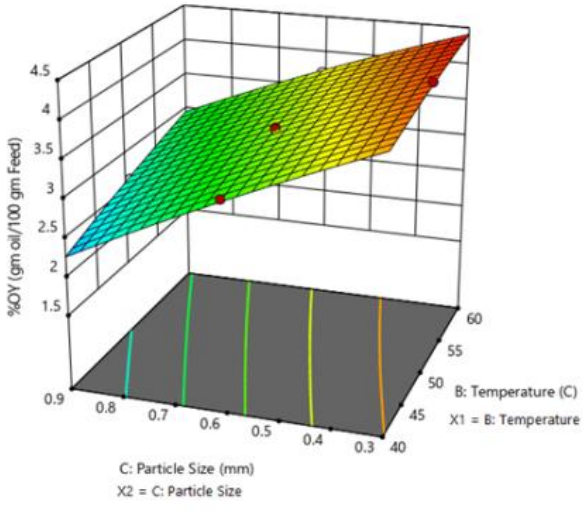

Fig. 3(c)

Fig. 3 Response surface plots (a-c) for turmeric oil
Fig. 3(a) percent yield vs. extraction temperature and pressure at a constant particle size of 0.6 mm;
Fig. 3(b) percent yield vs. particle size and extraction pressure at a constant temperature of 50^0C
Fig. 3(c) percent yield vs. extraction particle size and extraction temperature at a constant pressure of 24.55 MPa

Fig. 4 is a graphical representation of the predicted response of ANOVA vs. actual response in terms of %OY obtained from various experiments of RSM study. Finally, numerical optimization of the operating variables was carried out to predict the optimal conditions of three factors towards the maximized yield of turmeric. The optimized values of 3 factors with corresponding with maximized %OY are reported in Table 7.

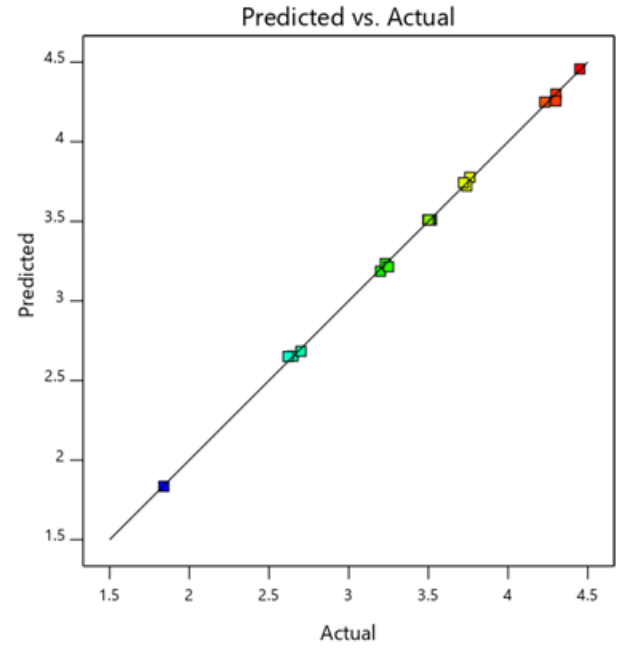

Fig. 4: Graphical representation of the predicted response of %OY vs. actual response of %OY

Table- 7 Optimum points of operating parameters to maximize the responses %OY

%OY	Pressure	Temperature	Particle Size
4.454	27.097	59.957	0.3

F. Chemical Analysis of Essential Oil Components of Turmeric Rhizomes

The volatile ingredients of the turmeric oil obtained from SCO_2E at optimal conditions using bed type AB1were analyzed. The full-length GC-MS chromatogram of turmeric oil was shown in Fig 5. The components identification methods were described in a previous study [25]. The identified compounds present in the turmeric oil sample were listed in Table 8. Some of the principal components detected are ar-Turmerone 57.21%, Curlone 14.63%, Curcumene 1.49%, Tumerone 1.21%, etc.

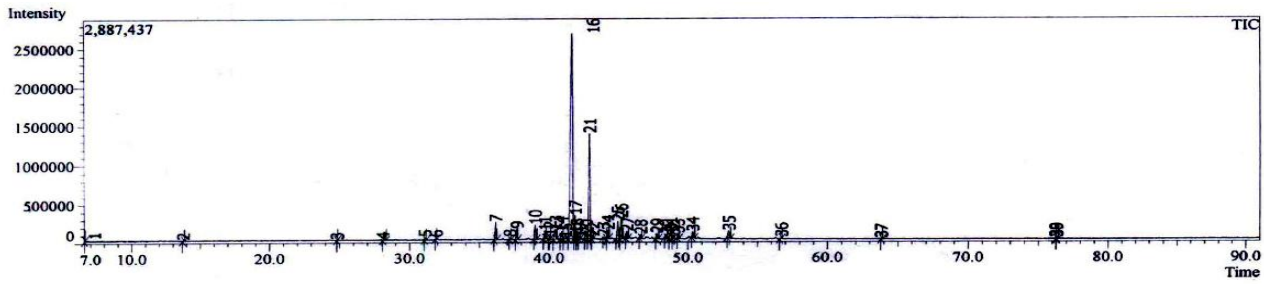

Fig 5: Gas chromatogram of bioactive components of turmeric essential oil

Table 8: Percentage chemical composition of the turmeric essential oil

Components Name	Molecular Formula	Molecular Weight	Retention Time	% Con.
Ethanol, 2-Methoxy-, Acetate	$C_5H_{10}O_3$	118.1311	6.551	0.27
ar-Turmerol	$C_{15}H_{22}O$	218.34	31.892	0.11
ar - Curcumene	$C_{15}H_{22}$	202.341	36.162	1.49
β-Sesquiphellandrene	$C_{15}H_{24}$	204.357	37.690	0.91
ar-Turmerol	$C_{15}H_{22}O$	218.34	39.012	2.05
ar-Turmerol	$C_{15}H_{22}O$	218.34	39.714	0.95
Lanceol	$C_{15}H_{24}O$	220.356	40.042	0.28
dihydro-ar-Turmerone	$C_{15}H_{22}O$	216.324	40.481	1.77
β-Biotol	$C_{15}H_{24}O$	220.356	40.910	1.18
Formic Acid, Benzoyl-,(8'-phenylmethyl) ester	$C_8H_8O_2$	136.15	41.250	0.29
ar Turmerone	$C_{15}H_{20}O$	216.324	41.680	57.21
Tumerone	$C_{15}H_{22}O$	218.34	41.868	1.21
1,5-Heptan-4-ol, 3,3,6-Trimethyl	$C_{10}H_{18}O$	154.253	41.995	0.61
β-Biotol	$C_{15}H_{24}O$	220.356	42.653	0.27
Curlone or β Tumerone	$C_{15}H_{22}O$	218.34	42.922	14.63
Turmerol	$C_{15}H_{22}O$	218.34	43.907	0.18
Bisabolone	$C_{15}H_{24}O$	220.356	44.257	1.31
ar-Turmerol	$C_{15}H_{22}O$	218.34	44.968	3.03
Atlantone	$C_{15}H_{22}O$	218.34	45.220	2.82
Atlantone	$C_{15}H_{22}O$	218.34	45.619	0.66
Tumerone	$C_{15}H_{22}O$	218.3346	46.603	0.43
Cyclohexane, (2-Nitro-2-Propenyl)	$C_9H_{15}NO_2$	169.2209	47.797	0.93
Atlantone	$C_{15}H_{22}O$	218.34	48.432	1.00
Cyclohexanecarboxylic acid, 3-phenylpropyl ester	$C_{16}H_{22}O_2$	246.35	48.787	1.03
Benzene, (1-cyclopenten-1-ylsulfonyl)	$C_{11}H_{12}O_2S$	208.277	48.975	0.30
α-Oxobisabolene	$C_{15}H_{24}O$	220.3505	49.317	0.88
5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	$C_{15}H_{26}O_2$	238.371	50.100	0.24
Atlantone	$C_{15}H_{22}O$	218.34	50.412	1.42
2-Methyl-4-octenal	$C_9H_{16}O$	140.226	52.967	1.47
Atlantone	$C_{15}H_{22}O$	218.34	56.374	0.18
2,5-Heptadien-4-one,2,6-Dimethyl-	$C_9H_{14}O$	138.21	56.759	0.26

Rest components were present in the range of 0.01 - 0.14.

G. Dynamic Mathematical Model of OECs

The OECs obtained from different experiments for RSM studies were found to fit in the Luo Denglin dynamic model type equation [36]. The model was expressed as-

$$Y = Y_{\propto}(1 - e^{-k.t})$$

where Y represents the amount oil extracted expressed as (%OY) at time t, $Y_{\propto}$ is a measure of the maximum value of Y after infinite time that is the maximum amount of extractable oil ($\%OY_{max}$) and k is a rate constant.

Y_{α} was substituted from the yield value obtained from Soxhlet extraction experiments.

The maximum oil yield obtained in the Soxhlet process was 5.42 ($which\ is\ equal\ to\ Y_{\propto}$). The rate constant, k, is found to be a function of reduced temperature and reduced pressure. It is defined as $\left[k = A.\frac{T/T_C}{P/P_C}\right]$,

where A is proportionality constant; T_C is critical temperature (31.1^0C) and P_C is critical pressure (7.39MPa) of solvent CO_2. The value of the constant 'A=0.025' was evaluated with the help of Curve Expert 1.40. The final dynamic equation was thus found to take the form:

$$Y = 5.42\left(1 - e^{-0.025\frac{T/T_C}{P/P_C}.t}\right)$$

It described all the OECs (obtained from the RSM study on turmeric oil extraction) quite well (with R^2 ranges from 0.9-0.99). Fig 5(a) and 5(b) show two sample of model fitting curves obtained under operating conditions of SFE of turmeric rhizomes (a) P= 21.6 MPa, T= 60^0C, and D_P=0.3 mm, and (b) P= 24.55 MPa, T= 50^0C, D_P=0.3mm, using annulus extractor AB1.

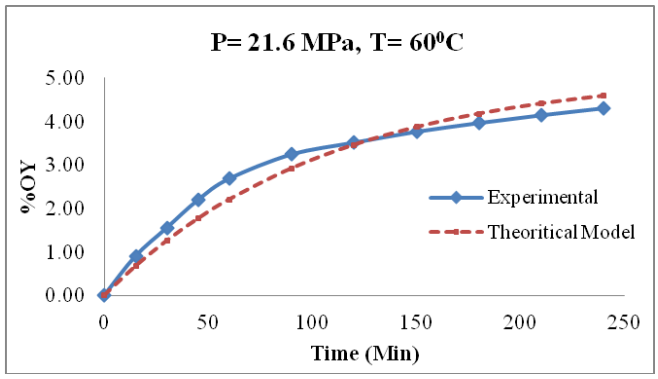

Fig. 5(a)

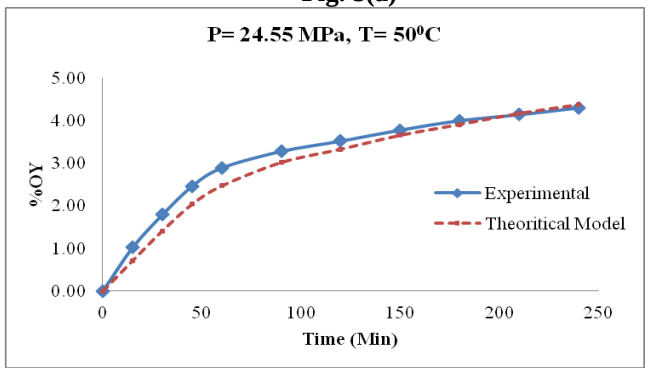

Fig. 5(b)

Fig 5 (a) & 5(b): Model Fitting Overall Extraction Curves of Turmeric Oil

IV. CONCLUSION AND FUTURE SCOPE

In the present study, turmeric oil was extracted from dried milled turmeric rhizomes using Soxhlet extraction and SCO_2E (without applying any co-solvent) methods. % OY obtained from the Soxhlet method was 5.42% and that obtained from SCO_2E (for an extraction period of four hours) was in the range of 1.84 to 4.45%.

Influence of individual process parameters like temperature, pressure, particle size and bed design geometry on turmeric oil-extract was studied thoroughly and the data were analyzed by using statistical response surface methodology (RSM). Annulus bed geometry shows a definite impact on the rate of extraction and %OY over conventional cylindrical geometry under the same operating conditions. Analysis of results of interaction plots indicates that pressure (in the range of 21.6-27.5 MPa) and temperature (in the range from 40^0C - 60^0C), both have a slightly positive impact on improving the %OY, whereas smaller particle size (0.3mm) has a significant effect on the %OY.

In RSM study using the FC-CCD method, a quadratic model exhibited the best fit to explore the relationship between operating parameters of SFE and the yield of turmeric rhizomes.

Chemical analysis of extract obtained from SFE applying optimum operating conditions and annulus bed geometry revealed the presence of 57.21% ar-Turmerone and 14.63% β –Turmerone, which indicates the good quality of the product.

Thus, it may be concluded that though the study of annular geometry in place of traditional cylindrical geometry of the extractor indicates an improvement of extractor performance in terms of increasing rate and yield of extraction for moderate extraction period or reduced extraction time to extract economically, further systematic studies with different bed geometries are necessary to correlate hydrodynamic behaviour for establishing a suitable criterion that can be used to predict extractor performance along with its economic gain.

Acknowledgments

All the authors profusely acknowledge the grant of AICTE for the development of Supercritical Fluid Extraction facilities under MODROB scheme where all the research works were carried out. Part of the running experiments was financially supported by IIChE, Kolkata, India. We are also grateful to M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India for extending its support towards GC-MS analysis of turmeric oil samples free of cost.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AB	Annulus Bed
CB	Conventional Cylindrical Bed
CCD	Central composite design
CER	Constant extraction rate
D_P	Particle size
FC-CCD	Face centered central composite design
GCMS	Gas chromatograph mass spectrometer
OEC	Overall extraction curve
% OY	Percentage of oil yield
Q_{CO2}	Solvent flow rate
RSM	Response surface methodology
SCO_2	Supercritical CO_2
SCO_2E using CO_2	Supercritical fluid extraction technology
SFE	Supercritical fluid extraction
t_E	Period of Extraction
t_S	Static period of extraction

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Supercritical Carbon Dioxide Extraction of Clove (Syzygium Aromaticum) Bud Oil in an Annular Grate Feed Contactor: Effect of Axial To Radial Surface Enhancement Factor

Sutapa Roy, Chandan Guha, Asit Kumar Saha, Somak Jyoti Sahu

Abstract: ***Experimental investigations were carried out with and without incorporating a perforated concentric tube (diameter 0.75 cm or 0.15 cm with one blind end at the upstream side) within a typical cylindrical extraction bed. A new parameter, axial to radial surface enhancement factor, $[(r_o-r_i)/2L]$, was defined to correlate the effect of bed geometry with extraction performance. Supercritical CO_2 extraction of clove bud for essential oils was carried out with three different bed geometry at three different pressures (14.7, 19.6, & 24.5 MPa) and temperatures (35^0C, 40^0C & 45^0C) combination. Detailed analysis of the extraction rate data was presented and found to have two distinct rate periods, namely constant extraction rate period and falling extraction rate period with a small transition period in-between. Effects of bed geometry, temperature, and pressure on the oil yield were analyzed statistically using face centered central composite design technique. The optimum conditions were identified as 24.5MPa pressure, 44.72 °C temperature and bed type with 0.15cm perforated concentric tube with the optimal yield of 17.981.***

Keywords: Supercritical carbon dioxide extraction, Clove oil, central composite design, kinetic study, Surface enhancement factor.

I. INTRODUCTION

The ever increasing demand of essential oils is due to their scented biodegradable aromatic compounds used in various applications as flavouring agents in food, beverages, confectionery; pharmaceutical aids; aroma chemicals in aromatherapy; perfumery and cosmetic ingredients; "green pesticides" and insect repellents [1-11]. Worldwide the commercial interests for essential oil production, isolation of its components of potential interest are gradually growing along with searching more and more species enriched with bioactive green components and cultivating them collecting from their wild origin for expansion of world market of natural essential oils. India is one of the world's largest producer, consumer and exporter of essential oils [12, 13]. The favourable climatic conditions and quality soil suitable for the agricultural growth of aromatic plants, continuous development in science and technology and huge investment in processing and trading essential oils make India to play the dominant role in the world market of aroma producing plants and essential oils.

Over the years, extensive research works are going on for continuous growth of the Indian essential oils based economy by developing new agro and extraction technology-based, quality-monitoring, customer-centric, market-driven industry. Since quality of the product has been given great importance in recent times, supercritical fluid extraction (SFE) technology using CO_2 as a solvent is established as an efficient and effective novel technology for obtaining valuable bioactive compounds in more concentrated form than other traditional methods for various plant extracts [14-20]. The extract obtained by supercritical carbon dioxide extraction (SCO_2E) is free of any solvent impurity which is the most attracting factor for this method becoming popular overall traditional methods facing severe problems by environmental regulatory acts due to the use of organic, hazardous, toxic solvents in their methods [21].

SCO_2E system consists of an extraction unit, extractor, which is the heart of the system, is a high-pressure vessel where actual extraction operation of natural biomass is carried out. Geometrical parameters of extractor have a significant influence on the overall extraction curve (OEC). Most of the research work in this field have been focused on mass transfer rate; phase equlibria; effect of various parameters such as temperature, pressure, particle size, solvent flow rate, maturity of biomass; characterisation of the extracts; and cost of extraction [22-26]. Effect of bed geometry on extractor performance in terms of bed volume, bed height to diameter ratio and related cost analysis were reported in some literature [27-31]. Still, bed geometry is gaining interest of researchers for enormous studies on the impact of bed geometry on extraction kinetics and scale up of SFE processes.

In this research work, a unique concept is introduced in the extractor bed geometry modifying the conventional pattern and effects of temperature and pressure on extraction of clove bud essential oil in this modified bed geometry were studied.

Revised Manuscript Received on April 06, 2019.

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At the same time effects of bed geometry on extraction kinetics were studied comparing the extraction curves. Clove is chosen as raw material considering various factors. (i) Among 10% essential oil bearing plants from giant plant kingdom most contain very low quantity barely exceeding 1% essential oil [32]. High essential oil content (more than 10%) is found in very few plants species. Clove (*Syzygium aromaticum*) and nutmeg (*Myristica fragrans*) are common examples in this category. Reports say that aromatic oil content of clove buds varies in the range of 15-20% [33]. (ii) Clove bud essential oil is ease to separate from its matrices applying moderate pressure and temperature (reported 40^0C and 15MPa by Prado et. al., 2011[34]) by SCO_2E compared to many more plant materials (suffering from high-temperature, high-pressure operations) and better quality product is possible to obtain using this technology [22]. (iii) Clove is champion among all spices. It has wide range of applications in food, cosmetic, and pharmaceutical industries for its major bioactive components eugenol, β-caryophyllene and α-humulene etc. as antioxidant, antimicrobial, antibacterial, anti-inflammatory, antiviral, antifungal, anti-diabetic, anti-carcinogenic, anesthetic, antiseptic, analgesic, etc. [33, 35-37]. Thus, selection of clove buds as "model raw material" is ideal for SCO_2E studies.

II. MATERIALS AND METHODS

A. Raw Materials and Chemicals

The quality flower buds of clove as available in local market were purchased from Haldia (West Bengal, India), checked thoroughly for removal of foreign materials (if any) and dried at 30°C in a laboratory scale air circulated drying unit for 12 h, in order to control the moisture content of the feed mass below 12% to avoid the impact of moisture on the volatile matter, mass transfer rate and solubility of the extract in the solvent CO_2 [38, 39].

The air dried clove sample was then ground into smaller particles in a mixture grinder (Philips Mixer Grinder HL7720) and stored in a cold place in air-tight containers for subsequent experimental studies on SCO_2E. The ground material was classified with sieves of mesh sizes 16 -42 from Tyler standard screen series. The particle size (D_p) of 0.64mm was obtained following the mass mean diameter calculation.

CO_2 (Commercial grade with above 99% purity) was purchased from Bharat Oxytech Pvt. Ltd., Haldia (West Bengal, India) and used as solvent for the SFE.

B. Moisture Content of Clove Buds

The moisture content of ground material was determined using the "SARTORIUS MA45C" moisture analyzer before and after drying. The moisture analysis results were prepared after triplicate measurements.

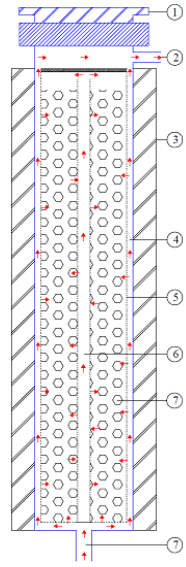
C. Determination of the Total Amount of Extractable Material (Global Yield)

The total extractable essential oil content from clove buds was determined using conventional solvent extraction method. To perform the experiment 30 gm dried ground clove bud sample from the same feedstock, as used for SCO_2E studies, was wrapped in a filter paper and loaded in a glass thimble which was connected with 500 mL capacity reflux flux connected with Soxhlet apparatus. Extraction was carried out using 300 mL n-hexane for 6hrs. The clove oil extract was concentrated by removal of solvent at 50^0C in a rotary vacuum evaporator.

D. Experimental Set-up and SFE Process

Extraction operations of clove buds essential oil were experimentally performed using a semi batch flow type SFE module (Model No: CSL/SCF/1L2/400) purchased from M/s Chemtron Science Laboratories Pvt. Ltd., Navi Mumbai, India. It is equipped with one high-pressure pump for solvent delivery (HP DOSING *PUMP* MODEL# *UMBL-30:* PLUNGER), one supercritical CO_2 generator, two parallel connected 1L Extraction vessels (each of 42cm height and 5.5cm inside diameter with maximum operable pressure of 29.42Mpa), and two low pressure Separators (each of 1L capacity) connected in series, and a refrigeration unit (design lowest temperature -14^0C). The extraction vessels, supercritical fluid generator and separators all are covered with heating jackets to keep temperature constant to the set point.

The solid feed samples were fed inside the extractor with the help of an externally loaded cylindrical shell having perforated surface. In this study a special modification in bed geometry was introduced by inserting a perforated small diameter concentric tube (with one blind end at the upstream side) inside this external cylindrical shell and ground clove buds was charged in the annular space of the modified bed. Two different diameters (0.75cm and 1.5cm) were chosen to design the annular bed geometry. The influence of annular solid beds (B2, designed using 0.75cm diameter inside channel & B3, designed using 1.5cm diameter inside channel) and conventional cylindrical solid bed without any internal annular path (B1) were studied under varying operating conditions. The diagram of an annular solid feed bed loaded inside the extractor is shown in Fig.1.



1. Extractor cap
2. Solute (essential oil) rich supercritical carbon dioxide
3. Thermostatic water bath
4. Extractor vessel
5. Perforated annular feed bed
6. Perforated concentric tube
7. Ground botanical mass
8. Supercritical carbon dioxide

Fig. 1: Supercritical Fluid Extractor Bed Design

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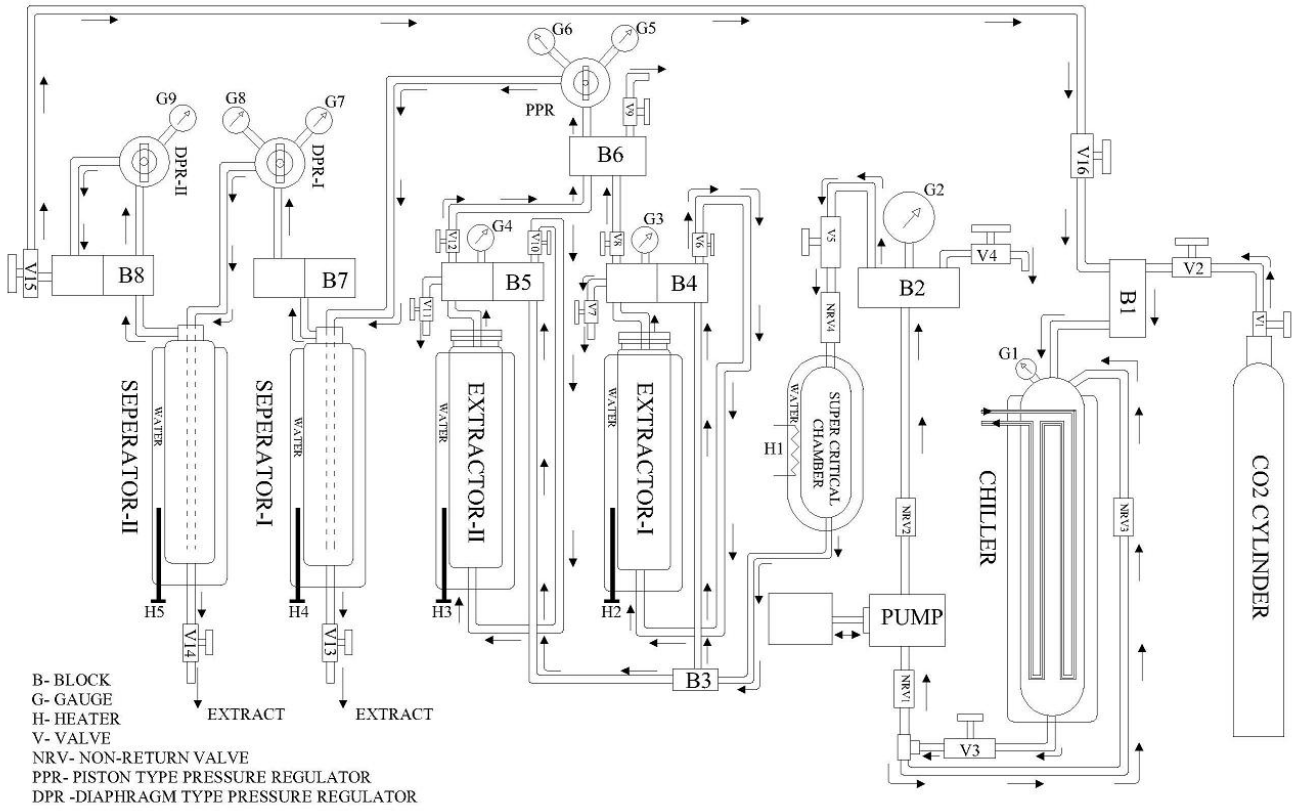

Fig. 2: Schematic of Supercritical Fluid Extraction Setup

For experiment initially a particular type of extractor bed among above mentioned three types were chosen and packed with crushed clove powder while both ends of the bed were covered with polypropylene wool to prevent any solid loss and protect the extractor lines from blockage. This externally filled solid bed of feed was then loaded inside the extractor vessel to carry out extraction.

Schematic of SFE module used in the present study is shown in Fig. 2**.** (i) Solvent CO_2 is supplied from cylinder in liquid form (ii) and allowed to enter in a reservoir (≈6.37MPa) where its temperature reduces below 5^0C. (iii) Cold liquid CO_2 is then pressurized to desired operating pressure with the help of a high-pressure pump (maximum pressure limit 29.42 MPa). High-pressure pump head temperature is controlled with the help of a cold water circulation system through which cold water of 4^0C was circulated. (iv) Pressurized liquid CO_2 is heated up to attain the desired supercritical temperature (which is above critical temperature 31.1^0C) of extraction in the SCO_2 generator with the help of a thermostatic water bath. (v) Now it enters into the already charged extraction vessel where desired supercritical condition is maintained throughout the period of extraction. After introducing the solvent in the extractor, the outlet valve of the extractor is kept closed to provide a static extraction period (t_s) of 20 minutes before starting the dynamic extraction to ensure better contact between fluid-solid phases in all the experiments performed. In the extractor SCO_2 penetrates in the molecules of charged material (crushed Clove in this study) and extracts their essence. Desired flow rate of SCO_2 is maintained for 210 min (t_t) for all experiments. (vi) From extractor this extract rich solvent is allowed to pass through two successive steps of extract recovery vessels termed as Separator-I and separator-II. (vii) At extract recovery vessels, temperature and pressure are reduced in two steps to that extent (below 31.1^0C, and 7.4 MPa pressure) so that SCO_2 converted back into gaseous CO_2 using Manual Back Pressure Regulator. Thereby, solubility of extracted oil in the solvent CO_2 drops significantly and oil is separated and drained out from bottom of the separator vessels. In all experiments, the oil sample was collected and weighed at intervals using different sampling bottles and recorded to prepare OEC. (viii) After second stage of separation oil free CO_2 gas from the top of the separator-2 is re-circulated back to refrigeration section for reuse. (ix) After completion of extraction closing the inlet and outlet valves of extractor, it is vented to unload the oil extracted solid mass. (x) The total extract is centrifuged and separated from other co-extracts and kept under refrigeration until the analyses were carried out.

E. Experimental Design for Extraction

The extraction yield of essential oil and the presence of important bioactive ingredients in concentrated form in the extracted mass are influenced by various operating parameters, directly or indirectly, such as temperature, pressure, particle size, solvent flow rate, time of extraction, use of co-solvent, level of moisture in the feed, porosity of feed bed, extractor bed geometry etc. [40] and their effects on oil yield may be either independent or interactive [41]. In the present work modified extractor bed geometry was chosen as one important factor to study its effect on efficiently extracting clove oil.

To express annular bed geometry of feed material, a new dimensionless group, axial to radial surface enhancement factor (ARSEF) which is the ratio of axial surface [i.e., cross sectional area $\pi(r_o^2-r_i^2)$] to radial surface area [i.e., $2\pi L(r_o+r_i)$], is introduced as $((r_o - r_i)/2L)$, where r_o is the radius of outer cylinder and r_i is the radius of inner cylinder, and L is the length of the bed. Three parameters (i) bed geometry $X_1 = \frac{r_o - r_i}{2L}$, (ii) temperature (X2) and (iii) pressure (X3) were considered to study their effects after implementing this modified pattern of bed on percentage oil yield [%OY = (gm of oil extracted / 100 gm of feed)]. All the experiments were designed to conduct keeping other parameters such as particle size(D_P), mass of feed loaded (F), solvent flow rate (Q_{CO2}) and extraction time (t_E) constant at 0.64 mm, 600 gm, 18.5 gm/min and 210 minutes, respectively. The amounts of clove buds used in different runs were taken from same sample prepared initially and stored properly.

Table I: Three levels of selected variables chosen for FC-CCD under RSM

ARSEF, $X_1 = \frac{r_o - r_i}{2L}$	Temperature, X2 (^{0}C)	Pressure, X3 (MPa)
0.0238 (-1)	35 (-1)	14.7 (-1)
0.0283 (0)	40 (0)	19.6 (0)
0.0327 (+1)	45 (+1)	24.5 (+1)

In this investigation, a statistical model was proposed to optimize the above mentioned three parameters for SCO_2E of clove buds oil. The statistical optimization procedures had huge applications in this field since it considers various possible interactions of variables during optimization [42]. Face centered central composite design (FC-CCD) was applied as an experimental design strategy to study the influence of these three factors on the %OY as response. In statistical design and analysis, central composite designs (CCD) are experimental designs under Response Surface Methodology (RSM), a powerful tool for process optimization [41, 43-45, 26]. Three independent variables (X1, X2, and X3) were tabulated at three levels which are generally coded as (−1), (0) and (+1). Twenty different combinations of these three independent variables are generated in face centered FC-CCD. The temperature and pressure conditions selected were 35^{0}C (-1), 40^{0}C (0), and 45°C (+1) and 14.7 MPa (-1), 19.6 MPa (0), and 24.5 MPa (+1), respectively. The minimum temperature (35°C) was chosen considering the critical temperature of the CO_2 (31.1°C) and the maximum (45°C) was set following the optimum temperature (40^{0}C) reported in earlier works [34] as well as to avoid extraction of unwanted compounds and possible thermal degradation of the extract at high temperature. Regarding the operating pressure, the minimum pressure of 150 bar was chosen to obtain a high solvation power related proportionally with density of CO_2 at all levels [46, 47] and a maximum value of (250 bar) was selected according to the study of Mukhopadhyay and Rajeev, 1998 [48]. Three values of the dimensionless parameter X1 used in this study were 0.0238(-1) for annular bed (B3), 0.0283 for annular bed (B2) and 0.0327 for conventional cylindrical feed bed (B1). Three levels of each of these three variables are represented here by Table I. All the 20 set experiments based on CCD combinations were performed and responses (% OY) were recorded and the analysis of variance (ANOVA) of each independent factor was performed using Design Expert-11 statistical package [43]. Thus, the influence of each independent factor and their interactions were examined and estimated statistically.

F. Kinetics of Clove Oil Extraction in Annular Feed Bed Using SCO2E

Extractor feed bed geometry is an important factor in the design and scale-up of a SFE process. Success of scale up procedure depends on the quality data of kinetic study obtained from OECs at lab scale or pilot plant scale experimental set up. In this study three different feed beds (B1, B2 and B3 as mentioned in section D) with two types of bed geometries were used. Thus the evaluation and comparisons of OECs for all these three bed geometries are very much relevant for future scale up.

All the experiments to develop OECs were conducted for three different beds (B1, B2 and B3) for three different temperatures (T1=35^{0}C, T2=40^{0}C, and T3=45^{0}C) using the same experimental set up used in FC-CCD study. Other parameters such as mass of feed loaded (F), extraction pressure (P), Particle size(D_P), solvent flow (Q_{CO2}), initial static period of extraction (t_S) and extraction time (t_E) were kept constant for all runs. For each run, 15 samples of clove oil were collected initially at an interval of 10 minutes up to 90 minutes followed by 20 minutes interval up to 210 minutes. All the experiments were performed twice in similar conditions. The experimental data are provided in Table II. The yield was expressed as %OY as mentioned in section E. The OECs obtained from various experiments were adjusted with two straight lines and mentioned as constant extraction rate (CER) and falling extraction rate (FER) periods using graphical method [29]. Kinetic parameters like (i) constant extraction period (t_{CER}), (ii) the rate of extraction during CER (R_{CER}), (iii) % yield achieved during CER (Y_{CER}), and (iv) percentage of total yield (%OY) were presented adjusting the OECs to compare the curves obtained from various feed bed geometry [34].

Table II: Experimental data of kinetics studies

F (gm)	Q_{CO2} gm/min	T in E (C^0)	P in E (MPa)	T in S_1 (C^0)	P in S_1 (MPa)	T in S_2 (C^0)	P in S_2 (MPa)	t_s (min)	t_E (min)
600	18.5	35/40/45	19.6	33	≈6	28	≈5	20	210

F - Mass of feed, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, E – Extractor, S_1 – Separator1, S_2 – Separator2, t_s - Static period, t_E – Extraction time

G. Clove Oil Characterisation by GC/MS analysis

Qualitative and quantitative analysis of essential oil of clove buds was performed to identify the components present in it based on chromatographic with a flame ionization detector and spectroscopic criteria. In present work *GCMS*-QP2010 SE (SHIMADZU, Kyoto, Japan), an advanced standard gas chromatograph *mass* spectrometer, was used for this analysis. It was equipped with DB - 1 MS UI capillary column (length 60m, inside diameter 0.25m, internal film width 0.25µm) for separating the components supplied by Agilent. The essential oil sample of clove buds was diluted using acetone in 1:4 ratios (1% oil and 4% acetone) for chromatographic injection with the help of auto injector. 1 µL volume of sample was injected in the split mode (1:50). The carrier gas Helium (He) flowed maintaining the flow conditions of (i) total flow 53.3 mL/min, (ii) column flow 0.50 mL/min, (iii) purge flow 3.0 mL/min and (iv) pressure 63.3kPa. Column oven temperature was gradually increasing from 50^0C, maintained for 3min, and then increased gradually at the rate of 1^0C min^{-1} for 10 minutes, 2^0C min^{-1} for 40 minutes and 3^0C min^{-1} for 30 minutes until 230^0C, maintaining isothermal condition for last 7 minutes. The total run time was 90 min.

Mass spectroscopic detector settings used were – (i) Ion source temperature 220^0C, (ii) interface temperature 300^0C. The obtained mass spectra can be matched using GCMS solution software (version 4) developed with MS library - NIST, Wiley, and SHIM. All the testing of samples was done in quality control laboratory of M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

III. RESULTS AND DISCUSSIONS

A. Moisture content and Global Yield :

Moisture content of raw sample of clove buds purchased from market was 13.25%. Moisture content of feed sample used for the extraction after drying and grounding was 6.83%. The global yield obtained from Soxhlet extraction procedure was 19%.

B. Analysis of Variance (ANNOVA)

Based on the FC-CCD combinations for three process variables (X1, X2, and X3) of SCO_2E of clove buds essential oils, the results of all runs (i.e. responses of FC-CCD) in terms of %OY and corresponding values of independent factors were reported in Table III. For fitting suitable model, all experimental data were analysed for linear, two-factor interaction (2FI) and quadratic models for the responses %OY based on R^2 [49], standard deviation, adjusted R^2, predicted R^2, "PRESS" value F-values, p-values and lack-of-fit tests results. The quadratic model presented highest F-value (205.23) and p-value < 0.0001 i.e. the quadratic model showed insignificant lack of fit for data. This model came out as best for exhibiting low value of standard deviation (0.0854), high value of R^2 (0.9978) [50], lowest value of "PRESS" and maximized Adjusted R^2 (0.9958) and Predicted R^2 (0.9659) [having a difference less than 0.2] among all above mentioned model [41,43,44].

Table III: FC-CCD data of three independent variables with their coded levels and response as percentage oil yields (% OY) of SCO_2E of clove buds

Run No.	Input Factors			Response
	ARSEF, $X_1 = \frac{r_o - r_i}{2L}$	Temperature, X2 (^{0} C)	Pressure,X3 (MPa)	%OY (g Oil/ 100 g Feed)
1	0.0283	45	19.6	16.42
2	0.0238	35	24.5	15.92
3	0.0283	40	19.6	16.07
4	0.0283	40	24.5	16.83
5	0.0327	35	24.5	13.51
6	0.0238	45	24.5	18.05
7	0.0327	40	19.6	14.55
8	0.0283	40	19.6	16.07
9	0.0283	40	19.6	16.07
10	0.0238	40	19.6	16.81
11	0.0327	45	14.7	14.13
12	0.0283	40	19.6	16.07
13	0.0283	40	14.7	15.03
14	0.0238	35	14.7	14.52
15	0.0238	45	14.7	15.79
16	0.0283	35	19.6	14.74
17	0.0327	45	24.5	15.85
18	0.0327	35	14.7	12.11
19	0.0283	40	19.6	16.07
20	0.0283	40	19.6	16.07

X1, ARSEF; X2, Extraction Temperature; X3, Extraction Pressure; %OY, Percent Oil Yield of Clove Buds

Finally, an ANNOVA test was performed for in-depth statistical studies of each process variables on the response for the selected quadratic model. The results of ANNOVA for response surface quadratic equation were illustrated in Table IV. High Model F-value of 503.32 suggested the response surface quadratic model is significant. P-values of the individual terms of the quadratic model equation less than 0.0500 indicate model terms are significant. Thus in this analysis X1, X2, X3, X1X2, X1X3, X2X3, $X1^2$, $X2^2$,$X3^2$ are all significant model terms.

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On the other hand, the value of adequate precision, a measure of signal to noise ratio, greater than 4 is desirable towards perfect fitting. An adequate precision value of 95.881 obtained in ANNOVA indicates the selected quadratic model adequately described the true behaviour of the system in comparison to the linear model and 2FI models.

C. Model Equation obtained from RSM

The quadratic mathematical model representing the percentage oil yield (% OY) as clove buds within a function of the three independent variables of present study on SCO_2E of the range of their values under investigation can be expressed by the following generalised equation:

$$\%OY = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X3 + \beta_{12} X1X2 + \beta_{13} X1X3 + \beta_{23} X2X3 + \beta_{11} X1^2 + \beta_{22} X2^2 + \beta_{33} X3^2$$

where %OY is the actual response; β_0 is the regression coefficient of intercept; β_1, β_2 and β_3 are the regression coefficients for linear fit; β_{12}, β_{13} and β_{23} are the regression coefficients for FI fit; and β_{11}, β_{22} and β_{33} are the regression coefficients for quadratic fit.

In terms of actual values of the regression coefficients the final regression model is given by:

$$\%OY = -31.02006 + (7.793\times10^{2})\, X1 + 1.562\, X2 + 0.289\, X3 + 5.393\, X1X2 - 3.096 X1X3 + (6.02\times10^{-3})\, X2X3 - (20.888\times10^{3})\, X1^2 - (20.545\times10^{-3})\, X2^2 - (6.815\times10^{-3})\, X3^2$$

Table IV: ANOVA for the clove buds SFE yield (% OY) in the FC-CCD

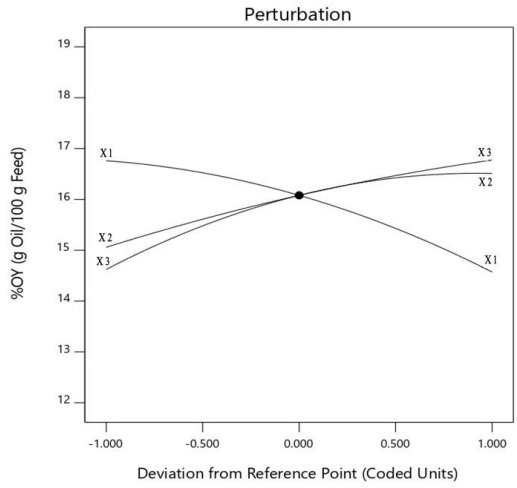
Source	Actual Regression Coefficients	Sum of Squares	df	Mean Square	F-value	p-value
Model		33.06	9	3.67	503.32	< 0.0001
X1	779.27810	11.97	1	11.97	1639.87	< 0.0001
X2	1.56208	8.91	1	8.91	1221.01	< 0.0001
X3	0.288898	7.36	1	7.36	1008.67	< 0.0001
X1 X2	5.39326	0.1152	1	0.1152	15.78	0.0026
X1 X3	-3.09562	0.0365	1	0.0365	4.99	0.0494
X2 X3	0.006020	0.174	1	0.174	23.85	0.0006
$X1^2$	-20888.088	0.4705	1	0.4705	64.47	< 0.0001
$X2^2$	-0.020545	0.7255	1	0.7255	99.41	< 0.0001
$X3^2$	-0.006815	0.0736	1	0.0736	10.09	0.0099
Residual		0.073	10	0.0073		
Lack of Fit		0.073	5	0.0146		
Pure Error		0.000	5	0.000		
Cor Total		33.13	19			
Std. Dev.	0.0854					
R^2	0.9978					
Adjusted R^2	0.9958					
Predicted R^2	0.9659					
Adeq Precision	95.8806					

X1, X2 and X3 relates the effects of main process variables ARSEF (r_o-r_i/2L), temperature (°C), and pressure (MPa), respectively on response (%OY). $X1^2$, $X2^2$ and $X3^2$ represents the quadratic affects of same input variables. X1X2, X1X3 and X2X3 are the interaction effects of three possible combinations of three variables (i) ARSEF and temperature; (ii) ARSEF and pressure , and (iii) temperature and pressure, respectively.

D. Effects of Bed Geometry, Temperature, and Pressure on the Oil Yield

Perturbation plot (Fig. 3) and two-factor interaction Response Surface plots Fig. 4(a-c) can be used to explain the effect of individual parameter as well as their interactions on the yield of extraction of clove buds in the range of values chosen for investigation. The Perturbation plot indicates the clove oil extracted by SCO_2E increases with increasing temperature from 35^0C-45^0C, initially at an increasing order from 35^0C-40^0C and after that with a decreasing order above 40^0C. This may be described by the counter effects of temperatures on solute and solvent of SFE. Temperature rise on one hand, improves the mass transfer rate of solute oil to the solvent phase by increasing diffusivity and vapour pressure values [51, 52, 40] and on the other hand, reduces the dissolving power of the solvent due to decreasing density. Temperature-solubility interferences are less significant at low temperature. At high temperature above it is more noticeable. Above 40^0C decreasing slope of %OY vs. temperature plot may be due to presence of this interference factor.

Similarly, Fig. 3 shows the pressure has an almost linear effect on oil yield at the range of pressure 14.7-24.5MPa [53]. P-value obtained from ANNOVA the diffusivity enhancing characteristics for the factor X3 representing extraction pressure was lowest and significant at 0.1% for linear fit as compared to the interactive and quadratic fits of X3 which were significant at 5%.



X1 : Bed Geometry Factor, X2 : Temperature (^{0} C),
X3 : Pressure (MPa)

Fig. 3. Effect of annular extractor bed geometry, temperature and pressure on the %OY of clove oil extracted by supercritical CO_2

This is well known that increase of pressure attributed to the increase of density of supercritical CO_2. With increasing density the interaction between solute molecules and CO_2 molecules increases due to decrease in intermolecular distances. Thus the dissolving power of CO_2 increases likewise liquid solvents and yield of oil increases [22, 54]. The adverse effects of increasing temperature on oil yields are insignificant up to 45^0C for clove buds [55].

Finally, the plot of %OY vs. factor X1 represents the influence of the modified annular extractor bed geometry over the conventional cylindrical bed type. Extract of SFE obtained from clove buds increases gradually and significantly from conventional bed to annular bed extractor containing larger size internal channel. Introduction of the annular feed bed should not oppose the pressure and temperature influences from their normal trends, rather increased the %OY for the same extraction time and same operating conditions. P-values of all linear, FI, and quadratic terms of X1 were significant in ANNOVA at 0.1%-5%.

The pressure–temperature interaction plot Fig.-4(a) shows increase in oil yield with increasing both the temperature as well as pressure in the range of 35-45^0C and 14.7-24.5KPa, respectively. Thus highest yield of clove buds by SFE was obtained at highest temperature –pressure point and lowest oil content was obtained at lowest point of the plot. It was observed that the solubility of bioactive components of essential oil depends on the balance between CO_2 density and vapour pressure of solute which in turn influenced by the extraction pressure and temperature. At low temperature and pressure the yield of oil increases with the increase of pressure at a given temperature due to less impact of temperature (up to about 45^0C) on solubility as compared to pressure [55]. Same way at low pressure the yield of oil increases with increasing temperature at a given pressure due to less effective negative impact of temperature on solubility as compared to more positive impact on vapour pressure and diffusivity [56].

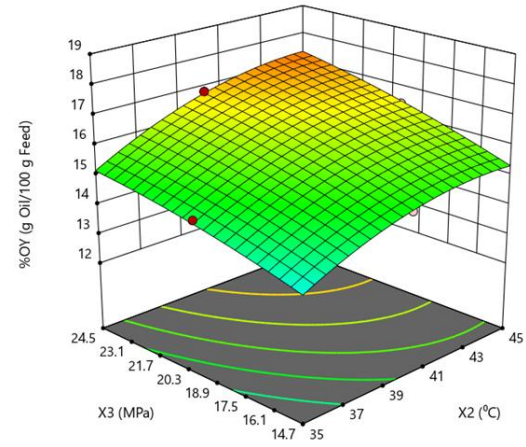


Fig. 4(a)

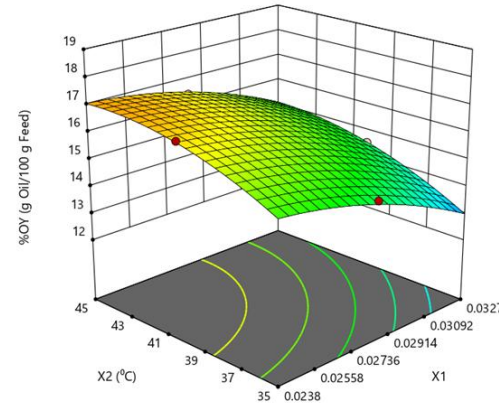


Fig. 4(b)

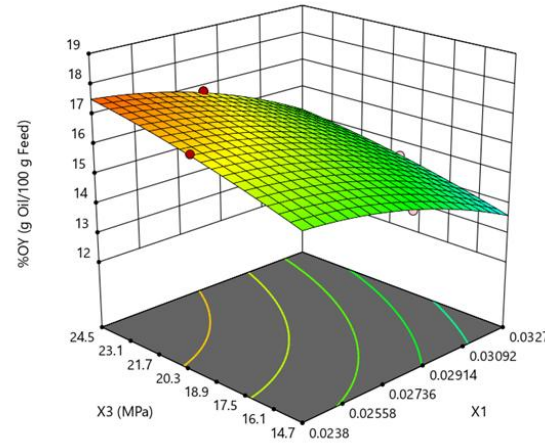


Fig. 4(c)

Fig. 4 Response surface plots (a-c) for clove oil:
Fig. 4(a) percent yield vs. extraction temperature and pressure at constant bed geometry (B2) ;
Fig. 4(b) percent yield vs. extraction temperature and bed geometry at constant pressure of 19.6MPa;
Fig. 4(c) percent yield vs. extraction pressure and bed geometry at constant temperature of 40°C

Fig. 4(b-c) show the three-dimensional plots of the response surfaces for the clove oil yield as related to extractor bed geometry parameter with temperatures as well as pressures. There, it is noticed that the increase of the ratio of radial to axial surface exhibit a positive effect on oil yield throughout the range of pressures and temperatures under this investigation. It is due to the fact that the increase of this ratio reduces the molecular diffusive path for both the solvent and solute molecules. This also induces turbulence in the fluid bulk that increases convective diffusion. As a result resistance to mass and heat transfer for oil extraction decreases yielding higher oil mass.

Fig. 5 represents the predicted response of ANNOVA vs. actual response in terms of %OY. Numerical optimization of the operating variables was carried out to predict the optimal condition in order to obtain the highest crude extraction yield of clove buds.

The optimum conditions were identified as 24.5MPa pressure, 44.72 °C temperature and bed type B3 with the optimal yield (%OY) of 17.981.

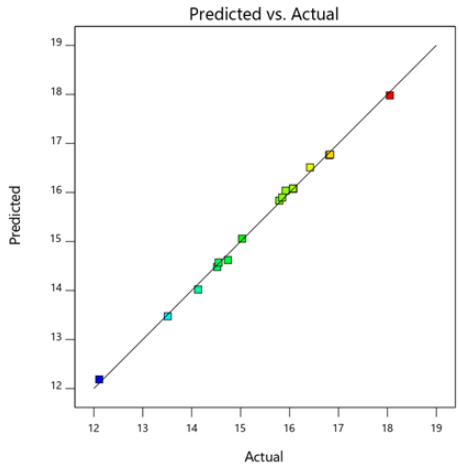


Fig. 5: Graphical representation of predicted response of %OY vs. actual response of %OY

E. Chemical Analysis of Essential Oil Components of Clove Buds

The chemical constituents of the clove oil obtained at 24.5MPa pressure, 45^{0}C temperature using bed type B3 by SCO_2E which was close to the optimum extraction condition of present RSM study were analyzed. The complete GC-MS chromatogram of clove oil sample is shown in Fig. 6. The components of clove essential oil were identified by comparing the retention times, mass fragmentation patterns of them with the available data of reference samples and GC-MS spectral database for organic compounds. The percent composition of essential oil constituents was determined using computerized normalization method from peak area of clove oil. Fig. 6 of chromatogram represents the presence several bioactive ingredients in the clove essential oil. The identified compounds present in the clove oil sample used for quantitative and qualitative analysis in this study were listed in Table V.

The main components identified in the clove extract were eugenol (72.08%), eugenyl acetate (11.84%), β-caryophyllene (6.73%), and caryophyllenoxide (3.06%). Roughly, the range of these constituents in good quality clove oil were reported as eugenol (70–95 %), eugenol acetate (up to 20 %) and β-caryophyllene (12–17 %) [33, 57, 58]. Main constituent eugenol percentage in this study match with the above mentioned range and indicates good quality oil. Presence of greater percentage of eugenol may be explained following the research work of Guan et. al., 2007 [22]. They reported in their work that the selective extraction of eugenol content is increased almost proportionally with temperature than other components. Clove oil analysed in this study was extracted using supercritical CO_2 at almost 45^{0}C which was almost 5^{0}C larger than the reported best extraction temperature by Prado et. al., 2011 [34].

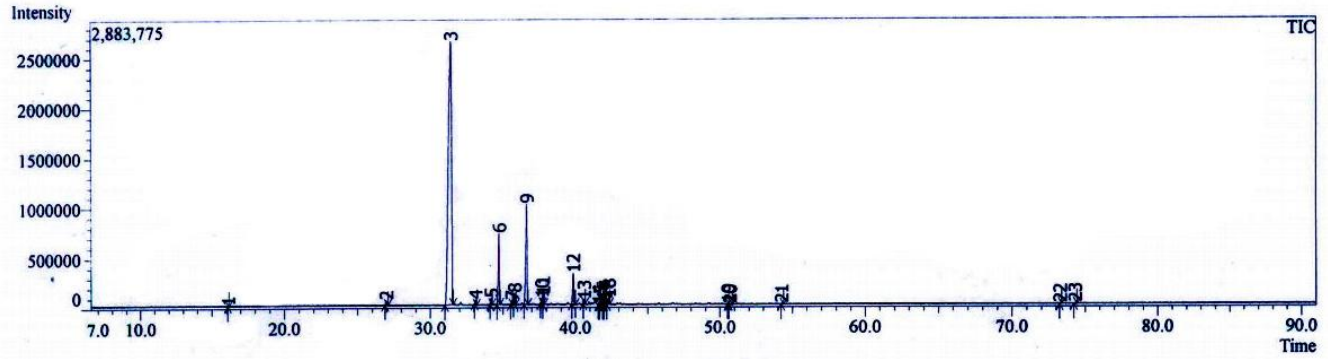


Fig. 6: Gas chromatogram of the constituents of essential oil extracted from clove buds

Table V: Percentage chemical composition of the volatile oil from clove buds

Compound Name	Mol. Wt.	Molecular Formula	Retention Time (Min)	% Conc.
P-Allylphenol	134	$C_9H_{10}O$	27.083	0.44
Eugenol NP	164	$C_{10}H_{12}O_2$	31.441	72.08
α-Copaene(23.49)	204	$C_{15}H_{24}$	33.106	0.38
α-Caryophyllene	204	$C_{15}H_{24}$	34.175	0.53
β-caryophyllene	204	$C_{15}H_{24}$	34.709	6.73
Humulene-(V1)	204	$C_{15}H_{24}$	35.583	0.29
Caryophyllene	204	$C_{15}H_{24}$	35.801	0.88
Eugenyl acetate	206	$C_{12}H_{14}O_3$	36.62	11.84
Calamenene(Trans) 29.96	202	$C_{15}H_{22}$	37.667	0.73
Delta-Cadinene	204	$C_{15}H_{24}$	37.757	0.52
Caryophyllenoxide	220	$C_{15}H_{24}O$	39.845	3.06
Humulene Epoxide	220	$C_{15}H_{24}O$	40.591	0.38
Cembrene	272	$C_{20}H_{32}$	41.888	0.30
2´,3´,4´ Trimethanoxyacetophenone	210	$C_{11}H_{14}O_4$	42.043	0.49
Caryophylenoxide	220	$C_{15}H_{24}O$	42.284	0.63

Rest components were present in the range of 0.03% - 0.24%.

F. Kinetics of Extraction under varying Extractor Bed Geometry:

Fig. 7(a-c) show that the variation in extractor bed geometry influenced the extraction rate while other conditions of extraction were maintained constant.

From the feasibility study on economic evaluation of some commercial SCO_2E process, it is considered that extraction of plant material will be continued up to recovery of 90% of the solute part. The increased operational cost involved with the continuation of extraction to recover rest portion of the volatile matter is not viable with the increments in the amount of product. In OEC, information about the CER period is very much important because it represents extraction period with highest and constant rate and greater productivity. Thus CER influences remarkably on the manufacturing cost of volatile matter from plant material. The largest amount of extract in unit time is possible in this CER period because solute mass is easily accessible by the solvent CO_2 and dissolved in supercritical phase. Table VI provides information about kinetic study parameters during CER period in terms of t_{CER}, R_{CER}, and Y_{CER}. For all the three levels of temperature (T1, T2, T3), OECs differed among beds B1 (representing conventional cylindrical bed geometry), B2 and B3 (representing annular extractor bed geometry of different dimensions.

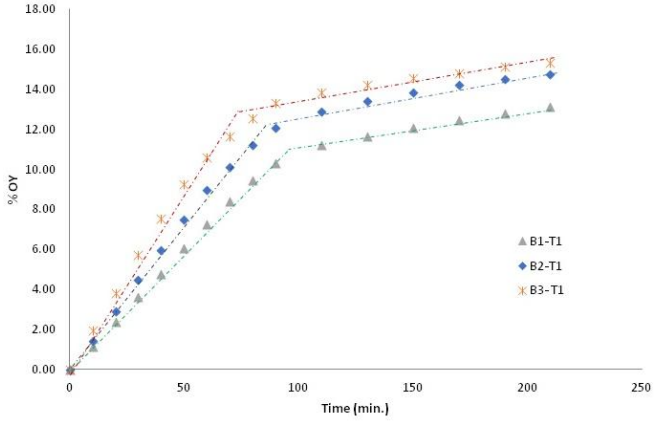


Fig. 7(a)

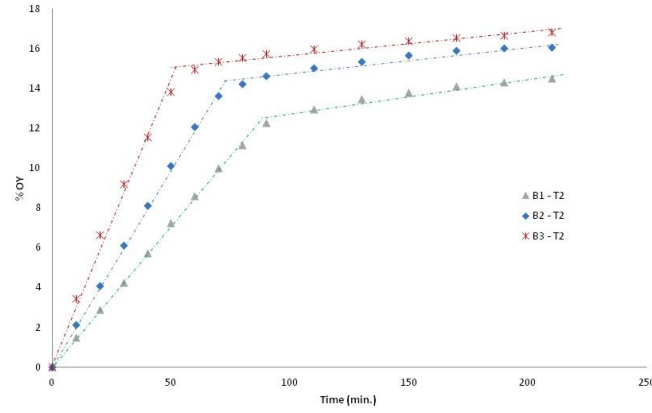


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Fig. 7(b)

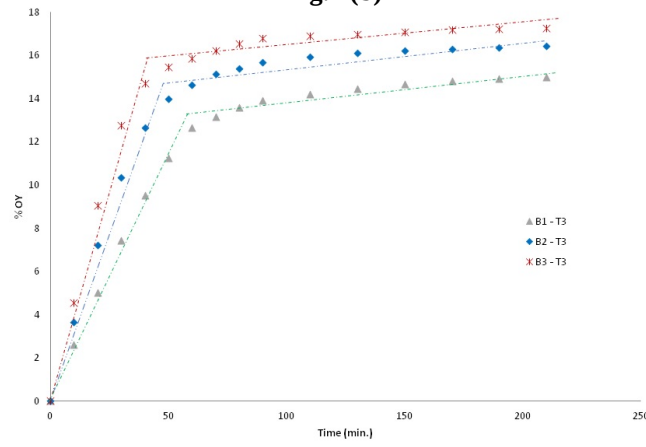

Fig. 7(c)

Fig. 7(a-c) Variation in OECs of clove buds oil under varying bed geometry B1, B2 and B3 for three different extraction temperatures of T1 (35^0C), T2 (40^0C), and T3 (45^0C), respectively.

In each bed with increasing temperature t_{CER} decreases, on the other hand R_{CER}, Y_{CER} and total yield (%OY) extracted in the period of 210 minutes increase. Positive effect of temperature on extraction of clove oil up to 45^0 C was reported in Guan et. al.,2007 research article [22]. Grosso et al. also reported that temperature helps to promote the faster release of the monoterpene hydrocarbons (main component of clove oil eugenol in this case) from the botanical materials [59]. It validates the increased values of R_{CER}, Y_{CER} during CER period. Comparative studies on the OEC curves of three beds reveal that t_{CER} is lowest in case of B3 type bed and highest in case of B1 type bed for all temperatures studied. On the other hand, R_{CER} and Y_{CER} are highest in case of B3 type bed and lowest in the case of B1 type bed for all temperatures studied. This happened due to the reduction of mass transfer resistance with the increase of the surface enhancement factor. As there is no depletion of solute molecules in the constant extraction rate period and surface diffusion controls the rate, the manifestation is quite prominent in CER rather than FER where pore diffusion controls the rate. From the rate curves it is also noted that though there increasing trends of R_{CER} and Y_{CER} from B1 to B3, it is not linear but of asymptotic in nature. This is quite obvious as larger voids invite ill effects of channeling.

Table VI: Experimental and estimated data for kinetic studies

Run	t_{CER} (min)	R_{CER} (10^{-6} kg/s)	Y_{CER} (%)	% OY (g Oil/ 100g Feed)
B1-T1	95.0	11.58	11.00	13.10
B2-T1	85.0	14.47	12.30	14.74
B3-T1	73.0	17.67	12.90	15.33
B1-T2	87.5	14.29	12.50	14.51
B2-T2	72.5	19.59	14.20	16.07
B3-T2	52.0	29.04	15.10	16.81
B1-T3	57.5	23.13	13.30	15.01
B2-T3	47.5	30.95	14.70	16.42
B3-T3	41.0	38.78	15.90	17.26

B1-T1, SCO_2E using extractor feed bed B1 with extraction temperature T1 (35^0C);
B2- T1, SCO_2E using extractor feed bed B2 with extraction temperature T1 (35^0C);
B3- T1, SCO_2E using extractor feed bed B3 with extraction temperature T1 (35^0C);
B1-T2, SCO_2E using extractor feed bed B1 with extraction temperature T2 (40^0C);
B2-T2, SCO_2E using extractor feed bed B2 with extraction temperature T2, (40^0C);
B3-T2, SCO_2E using extractor feed bed B3 with extraction temperature T2 (40^0C);
B1-T3, SCO_2E using extractor feed bed B1 with extraction temperature T3 (45^0C);
B2-T3, SCO_2E using extractor feed bed B2 with extraction temperature T3 (45^0C);
B3-T3, SCO_2E using extractor feed bed B3 with extraction temperature T3 (45^0C);
$t_{CER,}$ constant extraction rate period;
R_{CER}, the rate of extraction during CER;
Y_{CER},% yield achieved during CER;
%OY, percentage of total yield.

IV. CONCLUSION AND FUTURE SCOPE

The effects of bed geometry as axial to radial surface enhancement factor along with the common temperature-pressure parameters on the performance of supercritical carbon dioxide extraction of clove bud oil were studied and found to have definite advantages in terms of both the oil yields and time of extraction. The FC-CCD analysis of the results show that the clove oil extracted by SCO_2E increases with increasing temperature from 35^0C- 45^0C. But, the increasing tendency is not linear; rather initially it was of increasing order from 35^0C-40^0C and thereafter with a decreasing order. This may be due to the counter effect of temperatures on the solubility of solvent as the density of SCO_2 decreases with rising temperature.

The pressure has a positive effect on oil yield with almost linear with a slow rise in the range of pressures 14.7-24.5MPa. This is due to the increase of density of supercritical CO_2 with pressure and the interaction between solute molecules and CO_2 molecules increases with increasing density. Thus the dissolving power of CO_2 increases like conventional solvents and yield of oil increases. The effect of axial to radial surface area enhancement factor, $[(r_o-r_i)/2L]$, seems significant as extraction time reduces and oil yields increase with the increase of this factor. But after certain values of the enhancement factor, this became insignificant. This may be due to the fact that the channeling effect became pronounced at higher values of this enhancement factor.

Thus, it may be concluded that though axial to radial surface enhancement factor has a positive effect on SCO_2E, its efficacy after an optimal value gradually become flattened due to pronounce channeling effect. It is not clear at this stage, at least, how hydrodynamic behavior of the feed bed influences the extraction curve. Further systematic studies with different bed geometries in order to correlate hydrodynamic behavior are necessary for establishing a suitable criterion that can be used to predict extractor performance along with its economic gain.

ACKNOWLEDGEMENTS

This research work used the research facilities for supercritical fluid extraction developed under MODROB scheme of AICTE. The experimental works were financially supported by IIChE, Kolkata, India. GC-MS analysis of clove oil samples were done in the quality control laboratory of M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARSEF	Axial to radial surface enhancement factor
B	Bed type
CCD	Central composite design
CER	Constant extraction rate
D_P	Particle size
F	Mass of feed loaded
FC-CCD	Face centered central composite design

FER	Falling extraction rate
OEC	Overall extraction curve
OY	Percentage oil yield
Q_{CO2}	Solvent flow rate
R_{CER}	The rate of extraction during CER
RSM	Response surface methodology
SCO_2E	Supercritical fluid extraction technology using CO_2
SFE	Supercritical fluid extraction
SCO_2	Supercritical CO_2
T	Temperature
t_{CER}	Constant extraction rate period
t_E	Extraction time
t_S	Static period of extraction
Y_{CER}	% yield achieved during CER

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STUDIES ON REDUCTION OF EXTRACTION TIME OF SUPERCRITICAL CO_2 TO SAVE ENERGY

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Abstract

The present research work was conducted to examine the effect of changing geometric pattern of the extractor bed on the performance of supercritical co_2 extraction ($scco_2e$) of clove buds. The results of this study indicate that this modification of extractor bed geometry helps to complete this extraction process with higher yield in lesser time than conventional type of extractor bed. Since operating cost of extraction by supercritical fluid is higher than other methods, reduction of extraction time with better quality product in larger quantity significantly saves energy. $Scco_2e$ is already established as a promising novel technology for green extraction of natural products. Lot of research works are going on worldwide to reduce its energy consumption, thus cost of extraction to meet the challenges of the modern industrial development. This article explores a new research angle in the field of developing supercritical fluid extraction as an efficient, energy-saving, eco-friendly, sustainable technology for extraction of natural products from different plant materials.

Keywords: *Energy Saving, Green Extraction, Supercritical Co_2 Extraction, Extractor Bed Geometry.*

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1. INTRODUCTION

The challenges of the industrial development for green extraction of natural products like essential oils are multiple: more compact production units with reduced number of unit operations, developing innovative technologies with energy saving, cleaner and safer extraction protocols, limiting waste production, etc. Microwave-assisted extraction, ultrasound-assisted extraction,and supercritical fluid extractions (SCFE) are some of the novel techniques emerged in recent years for green extraction of natural products with a similar or better quality to that of traditional methods while reducing operational units thus helps to reduce total extraction time and save energy, minimize CO_2 emission and produce less waste [1,2]. From the point of reduction of stages, a single-stage process would appear to be ideal. The process of extraction by supercritical CO_2 ($SCCO_2E$) has this advantage. It uses no/very less amount of organic solvent(as co-solvent to extract polar component) and the time of extraction is very less than traditional extraction processes (like solvent extraction, distillation etc.) due to high selectivity, gas like diffusivity, liquid like solubility, high penetration rate of CO_2 in supercritical state [3,4]. Extract is completely solvent free as CO_2 is gas in atmospheric condition [4]. Use of CO_2 as solvent does not cause any additional green house effect as CO_2 is already produced in different processes, during respiration of animals and plants and present in the atmosphere. In conventional methods though extraction cost is less, most energy consumption occurred to recover the extract by evaporating the solvent and to eliminate residual solvent from the plant residue. Whereas in $SCCO_2E$ though extraction cost is higher but there is no solvent recovery energy cost. Yield and efficiency are also high in $SCCO_2E$ than above mentioned methods [3]. To reduce energy consumption technological modification may be possible in $SCCO_2E$ to recover the calories from conversion of CO_2 gas to liquid form and feed it into the heating system in the passage from liquid form to supercritical state. The efficiency of extraction methods need to be considered along with the cost of energy. This efficiency is based on the highest recovery of the effective constituents, the shortest processing time, use of minimum organic solvent, handling less amount of environmentally safe solvent. In this regard if the all steps of processes are taken into account, and not the individual extraction step, the $SCCO_2E$ has also emerged as an energy-saving technology.

In the present work $SCCO_2E$ of clove bud essential oil was conducted changing extractor bed geometry from the conventional packed bed geometry and its effect on extraction time and extraction yield were studied. Clove(scientific name *Syzygium aromaticum*) is one of the most important medicinal herbs and its bioactive components mainly eugenol, eugenol acetate, and β-caryophyllene possess antioxidant, antimicrobial, antifungal, anti-inflammatory, antiseptic, pain reliving and stimulating properties [8]. It is widely used in food, pharmaceutical, perfumery industries and aromatherapy. Comparative studies on clove oil extraction by different traditional methods and $SCCO_2E$ method established $SCCO_2E$ as the optimum extraction process for high quantity and high quality yield in shortest time [3]. To prevent channeling flow, to reduce dead space and for uniform distribution of the solvent use of glass beads along with solid sample was mentioned in literature [5,6]. The effect of length to diameter ratio of the extractor bed on SCF extraction was also provided in some literature [7]. But no previous references in literature are available where extractor bed geometry is directly changed to study its effect on essential

oil extraction. In the present article the comparisons have been performed in terms of the total yield of clove oil and extraction time for three different types of bed geometry. Results show that this modification of bed geometry decrease extraction time significantly and increase oil yield in case of a particular modified bed condition. Reduction of extraction time means reduction of energy cost. Thus this study is a promising step towards the supercritical fluid extractor design to meet the challenges of efficient energy saving technology.

2. MATERIALS AND METHODS

2.1 Materials

Dried clove buds were purchased from local market of Haldia, West Bengal, India. All clove samples were stored in room temperature protecting from sunlight in closed plastic container to prevent any loss of volatile matters before further processing. The solvent carbon dioxide of above 99% purity was obtained from Bharat Oxytech Pvt. Ltd.; Haldia, West Bengal, India.

2.2 Preparation Of Solid Feed Sample

Dried clove buds were ground by a grinder to increase surface area as well as to rupture the cell wall before every run and the particle size was measured by mechanical sieving after extraction. An average particle size of 0.70±0.05mm was obtained for most of the experiments.

2.3 Experimental Apparatus

Experimental set up for SCFE was supplied by Chemtron Science Laboratories Pvt. Ltd., Mahape, Navi Mumbai, India. Its main components include one positive displacement pump to pressurize solvent CO_2 to required operating pressure, SCF generation unit, two parallel connected high pressure Extractors (each of 50mm inside diameter, 500mm height), one pressure reduction valve, and two low pressure Separators connected in series to confirm complete separation of extract from solvent. Bed geometry was changed by inserting a cylindrical channel coaxially with the conventional cylindrical extractor bed. Two different diameter channels were used to check their effect on extraction separately along with the conventional packed bed.

2.4 Supercritical CO2 Extraction

Details about supercritical fluid extraction methods are available in different literatures and books. For each of the preset extraction test only one extractor among two was packed with dried, ground clove buds under three different conditions of bed geometric. Experiments were carried out for varying condition of temperature (315K and 318K) keeping other parameters like operating pressure (190 ± 10 kg/cm^2), CO_2 flow rate almost constant for all these three types of bed geometry. CO_2 after solvent conditioning was pressurized through a high-pressure pump to the required operating critical pressure and entered in the SCF generator to raise the temperature to a desired value. $SCCO_2$ was then allowed to enter the extractor charged with solid sample. The extract-laden CO_2 was sent to the separators via the pressure reduction valve to precipitate out the extract completely into the separators. CO_2 gas after releasing extract is recycled back for further use until extraction is completed. The oil weight was measured periodically until extraction is completed.

2.5 GC/MS analysis of Clove Oil

GC/MS analyses were performed in the laboratory of Chemical Engineering Department, IITG, Assam, India.

3. RESULTS AND DISCUSSIONS

Main objective of this work was to study the effect of introduction of coaxial channel inside the extractor bed on total extraction time and total oil yield to verify whether this new invention can reduce extraction time significantly to save energy without any compromise with the quality of extract. For all these experiments pressure, particle size and CO_2 flow rate were kept constant. Table-1 shows extraction time and % yield for varying operating conditions of temperature, type of bed geometry, weight of feed for each of the SCFE runs. Fig. 1 and Fig. 2 show the effect of modification of bed geometry on extraction time and total yield content at two different temperatures. It can be noticed from the results that maximum yield was obtained when extractor was operated at 318K temperature using larger diameter channel (15mm) inside the conventional packed bed. Extraction time was also reduced /saved by almost 36 minutes for this run. The increase of temperature results in increase of oil yield in all types of bed geometry. Use of annular channel without any glass beads for extraction of clove oil increases oil yield for both types of modified bed when compared with normal packed bed. As per the results of GC/MS analysis the main components of clove oil identified were eugenol (58%), β-caryophyllene(11%) and eugenol acetate(22%) which are in good agreement with some of the articles. The main cause behind the less time requirement for extraction along with better quality, large quantity yield content by this modified bed geometry is significant reduction of the resistance to mass transfer process which enhances contact between both phases thus increasing extraction rate significantly.

Table - 1: Experimental Results for Extraction of Clove Oil with SCCO2

Run No.	Extractor Bed type	Operating Temp (K)	Sample Weight (gm)	Extraction Time (min)	% Yield (gm oil /gm solid Feed)*100
1	Normal Packed Bed	318	450	203	11.00
2	Extractor Bed with Large Dia. Channel	318	450	167	16.40
3	Extractor Bed with Small Dia. Channel	318	450	199	15.00
4	Normal Packed Bed	313	250	99	8.60
5	Extractor Bed with Large Dia. Channel	313	250	110	13.35
6	Extractor Bed with Smalll Dia. Channel	313	250	163	11.00

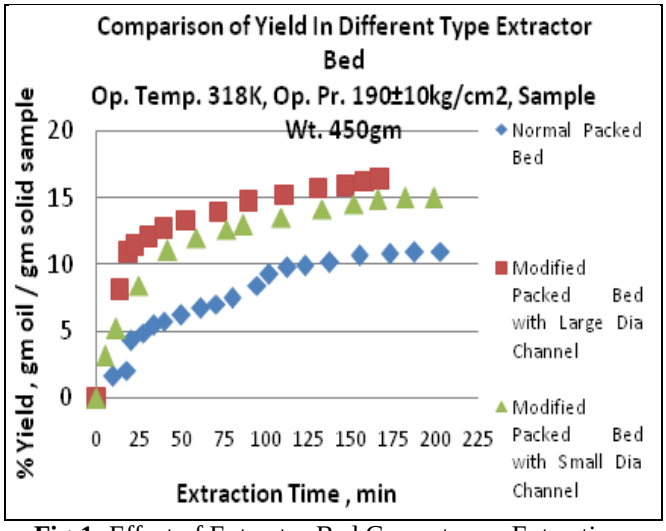

Fig-1: Effect of Extractor Bed Geometry on Extraction Time and yield Content

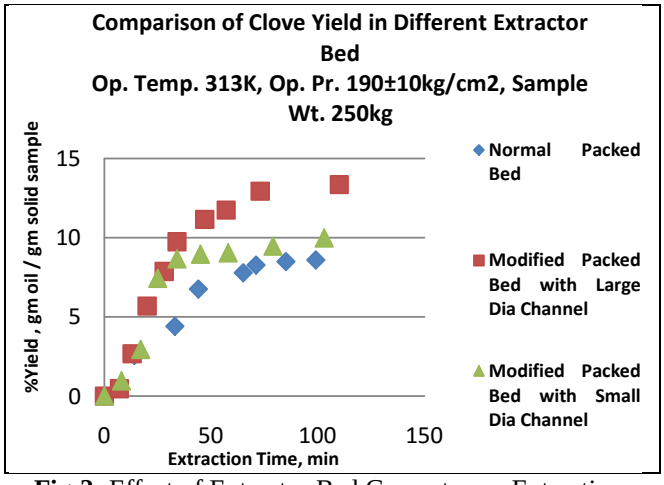

Fig-2: Effect of Extractor Bed Geometry on Extraction Time and yield Content

CONCLUSION

A modified static bed with varying annulus space was manufactured in this work to measure the yield of clove extract using supercritical CO2. The obtained yields with respect to time clearly indicate its edge over conventional fixed bed. Reduced extraction time with increased oil yield was indication of efficient extraction in modified bed compared to normal bed. The incorporation of perforated hollow cylinder in conventional fixed bed brings differences for the leached solids on accessibility, percolation and diffusion by the SCF. These factors can have a strong effect on improved yield and cost reduction.

ACKNOWLEDGEMENTS

This work was partly supported by AICTE under MODROB scheme to develop the laboratory for supercritical extraction. This work was financially supported by IIChE, Kolkata, India through project titled “Supercritical Fluid Extraction of Natural Products from Various Plant Resources”.

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Studies on Extractor Bed Geometry on Supercritical Carbon-dioxide Extraction of Clove Oil

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The supercritical fluid extraction (SFE) technology using CO_2 as a solvent is already established as an effective technology to produce high quality clove bud essential oil concentrated in eugenol and eugenyl acetate. The effect of different parameters, such as operating temperature, pressure, particle size, CO_2 flow rate , extractor bed height etc. are already reported in different research works. In the present work the effect of changing extractor bed geometry on the extraction yield and extraction time were studied. In place of cylindrical packed bed an annular packed bed concept was introduced. SFE of clove buds were conducted at 319K operating temperature, 210kg/cm^2 pressure of solvent for average particle size 0.75mm. The comparison has been performed in terms of the total yield of clove oil and extraction time for three different types of bed geometry. Results show that this modification of bed geometry has significant effect to improve extraction of clove oil by Supercritical Fluid. Using modified bed geometry total oil yield increases in the range of 2-5 % and extraction time also decreases. This change in bed geometry may be considered for supercritical fluid extractor design and economic study of the process.

Figure 1: Experimental SetUp

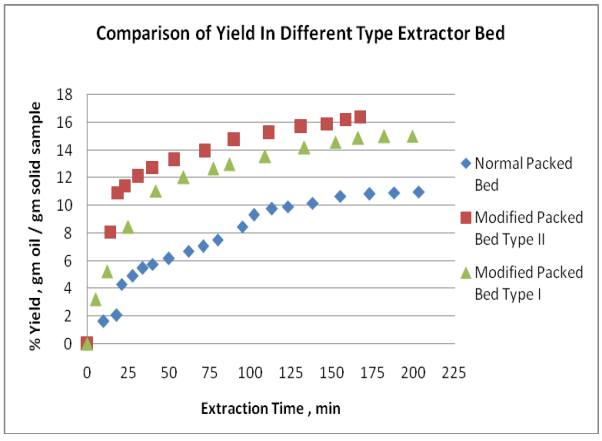

Figure 2: Extraction Curve

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Supercritical Fluid Extraction : A Green Extraction Technology to Protect Environment

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Abstract

With the growing consciousness of the consumers about health, quality and safety of foods and cosmetics the preference and demands for natural products as opposed tosynthetic substances have significantly been increased. In the conventional methods of extraction for natural products large amount of hazardous, carcinogenic, or toxic organic solvents are used. The high energy costs of solvent regeneration and the compelling regulations on the usage of these organic solvents to protect our environment and health are the main barriers towards the growth of the natural products industry. Accordingly, the development of green extraction technologies for the extraction of natural products to compete with the synthetic market becomes an interesting research topic. The main objectives of green extraction processes include the use of alternative safe solvents in place of organic solvents, minimization of extraction time, reduction of energy cost by energy recovery and other innovative techniques, elimination of multiple steps of unit operations, and production of biodegradable, safe and high quality contaminant free extract. Supercritical fluid extraction technologyis increasingly gaining importance as it is suggested by the researchers as a novel green extraction technology that can meet most of the above mentioned objectives. In this present work extraction of essential oils from natural plant materials were carried out using supercritical carbon-dioxide by designing an extractor to reduce the extraction time, increase yield, and produce organic contaminant free extract.

Keywords –Natural products, organic solvents, green extraction technology, energy recovery, supercritical fluid extraction.

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