Green solvent extraction, purification, carrier design and food application of natural antioxidants from ginger varieties of northeast India

A Thesis Submitted for the Award of the Degree

of

DOCTOR OF PHILOSOPHY

by

Abhishek Shukla

(Roll No. 136107006)

Department of Chemical Engineering

Indian Institute of Technology Guwahati

Guwahati-781 039, India

January 2021

TH-2386_136107006

TH-2386_136107006

Department of Chemical Engineering

Indian Institute of Technology Guwahati, Assam, India

STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemical Engineering, Indian Institute of Technology Guwahati, Assam, India under the supervision of **Prof. Vaibhav V. Goud** and **Prof. Chandan Das**

In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigations.

Guwahati

January 2021

Signature

TH-2386_136107006

TH-2386_136107006

Department of Chemical Engineering

Indian Institute of Technology Guwahati, Assam, India

CERTIFICATE

It is certified that the work contained in this thesis entitled "**Green solvent extraction, purification, carrier design and food application of natural antioxidants from ginger varieties of northeast India**" by Mr. Abhishek Shukla (Roll No. 136107006) for the award of Ph.D. degree, is a record of original research carried out by him at the Department of Chemical Engineering, Indian Institute of Technology Guwahati, under our guidance and supervision. The work embodied in this thesis has not been submitted to any other University or Institute for the award of any other degree or diploma.

4.3.21

Dr. Vaibhav V. Goud

Professor

Department of Chemical Engineering

Indian Institute of Technology Guwahati

Date:

04/03/2021

Dr. Chandan Das

Professor

Department of Chemical Engineering

Indian Institute of Technology Guwahati

Date:

TH-2386_136107006

TH-2386_136107006

ACKNOWLEDGEMENTS

It is my great privilege to sincerely thank the people who have supported me throughout my doctoral studies.

First and foremost, I take this opportunity to express my deep sense of respect and gratitude to my thesis supervisors, **Prof. Vaibhav V. Goud** and **Prof. Chandan Das** for providing inspiration and valuable guidance throughout the course. I am also indebted to them for their constant suggestions and encouragement. It has really been a remarkable experience working with them.

I would like to acknowledge my sincere gratitude to my doctoral committee members, Prof. G. Pugazhenthi, and Prof. Animes K. Golder, Department of Chemical Engineering and Prof. Pranab Ghosh, Department of Civil Engineering. Their strategic insights, valuable advice, and constructive suggestions during my research progress kept the flow of my research work in the right direction.

I would specially like to acknowledge my sincere gratitude to Prof. Ramgopal V. S. Uppaluri for his invaluable and continuous encouragement as well as moral and spiritual support during my research work.

I also thank Prof. Anugrah Singh, Head of Department of Chemical Engineering for his administrative support. Furthermore, I would like to thank all the other faculty and staff members specially Sailenda, Jayantoda, Arifulda, Balenda, Jyotishda and Chandan of my department. Deep gratitude for various support staff from academic and student affairs sections for their invaluable support during my doctoral studies.

I am grateful to the Analytical Laboratory of the Department of Chemical Engineering and Central Instruments Facility, IIT Guwahati, for providing me the necessary support for sample analysis.

I am thankful to Mr. K. C. S. Kurup (NERAMAC, Guwahati) for helping me in collecting ginger samples of different states of northeast India. I am also thankful to Mrs. Megokhono Meyase (Subject Matter Specialist, KVK Wokha), for her help in procurement ginger from Nagaland.

I would sincerely like to thank Acharya Balkrishna and Shri Rambhagat for providing me permission and necessary clearances to carry out experiments at M/s Patanjali Food & herbal Park, Haridwar. I would also like to express my deep gratitude to Dr. R. S. Shukla, New Product Development Department for his invaluable suggestions on commercial aspects of developing food products as well as his invaluable

TH-2386_136107006

insights on practical aspects of scale-up validation for large scale commercial manufacturing. I am also thankful to Dr. Nimai Ghosh and Shri D. S. Yadav for analytical facilities and employees of extraction plant as well as other support departments of M/s Patanjali Food & herbal Park, Haridwar.

I wish sincere gratitude to Prof. S. N. Naik from IIT Delhi for his constant support and allowing me to conduct experiments on his laboratory scale supercritical CO_2 apparatus. I would also like to thank Mr. Deepak Soota and Mr. Arun Mittal of M/s Proderna Biotech, Bhiwadi (India) for allowing me to conduct scale-up trials at their commercial scale supercritical CO_2 plant. Due acknowledgements are also to M/s India Glycols Limited, Dehradun for providing pure gingerols standard for analytical purpose.

I am sincerely thankful to Mr. Anurag Tyagi, Dr. Chanda Kapadia and Dr. Ketkee Deshmukh from M/s Gatteffosse India Pvt. Ltd., Mumbai for providing various surfactants and co-surfactants samples and their encouragement for carrier development studies.

I also wish to thank Prof. Arun Goyal, Department of Biosciences and Bioengineering for motivation and encouragement during the entire duration of my Ph.D.

I would always be indebted to my department friends Dr. Pallab Das, Dr. Rajeev Parmar, and Atanu Kumar Paul for helping and supporting me in every possible way throughout my Ph.D. life. I am also thankful to my lab mates Dr. Arijit Das, Dr. Sujoy Bose, Dr. Raj Kumar Das, Dr. Venkatnarasimha Rao Chelli, Dr. Suman Saha, Mr. Chitta Ranjan Barik, Mr. Abhradip Pal, Miss Mounika, Mr. Nagendra, Miss Sushma Chakraborty, and Mrs. Aritra Das for their support and co-operation.

Finally, my wholehearted gratitude goes to my family and in-laws for their blessings, and support as well as to my better-half Mrs. Asmita Shukla for her endless support, patience and wisdom which greatly eliminated obstacles and made my Ph.D. journey smooth, joyful and enjoyable.

Sincerely,

Abhishek Shukla

TH-2386_136107006

TABLE OF CONTENTS

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

Nomenclature

ρ_{avg}	Average ginger powder particle size
Q_{CO2}	$SCCO_2$ flow rate
S/F	Solvent to feed ratio
a_w	Water activity
k	Rate constant
R	Ideal gas constant (8.314 J/mol K)
T	Temperature (K)
A	Pressure (bar)
B	Flow rate (g min^{-1})
C	Time (min)
k_w	Moisture mass transfer coefficients
k_s	Solids mass transfer coefficients
k_g	Gingerols mass transfer coefficients
$x_{\infty w}$	Equilibrium moisture contents
$x_{\infty s}$	Equilibrium solids contents
$x_{\infty g}$	Equilibrium gingerols contents
D_{es}	Solid diffusion coefficients
D_{em}	Moisture diffusion coefficients
D_{eg}	Gingerols diffusion coefficients
k	Rate constant
D_e	Moisture diffusivity
r	Pearson's correlation coefficient

TH-2386_136107006

Abbreviations

$SCCO_2$	Supercritical CO_2
SMEDDS	Self-micro emulsifying drug delivery systems
RSM	Response surface methodology
QE	Quercetin equivalents
TFC	Total flavonoid contents
TPC	Total Phenolic content
RCCD	Rotatable central composite design
RSA	Radical scavenging activity
DPPH	2, 2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium
GAE	Gallic acid equivalent
OD	Osmotic dehydration
AC	Antioxidant capacity
PCA	Principal component analysis
HCA	Hierarchical cluster analysis
RDA	Recommended daily allowance
ΔE	Total color difference
BI	Browning index
WSI	Water solubility index
OSS	Overall sensory score
PET	Polyethylene terephthalate
MET	Aluminium metal-based multi-layered material
EVOH	Ethylene-vinyl alcohol
ASLT	Accelerated shelf-life testing
MASLT	Multivariate accelerated shelf-life testing
Sep	Separator
AOAC	Association of official analytical chemists

TH-2386_136107006

List of Figures

TH-2386_136107006

List of Figures

TH-2386_136107006

List of Figures

TH-2386_136107006

List of Figures

TH-2386_136107006

List of Tables

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

TH-2386_136107006

Abstract

Antioxidants play a vital role in preventing or delaying autoxidation and have attracted commercial interest as additives and stabilizers in food product development, dietary supplements, natural health products, and a range of pharmaceutical products. Northeast India is one of the largest biodiversity hotspot regions of the world. Ginger is one of the main cash crops of this region, hence the ginger varieties found in this region could potentially be one of the best varieties of the world. Ginger is abundantly rich in highly potent antioxidants that have been extensively studied for its wide range of health benefiting qualities. Owing to the distinct flavor, antioxidants of ginger are preferred templates for commercial application as a natural flavoring agent. Considering this as motivation, gaps were identified in the existing state of the art, and this research work has been undertaken towards exploring the antioxidant potential of northeast Indian ginger varieties and its food applications. The entire thesis work is divided into five major parts.

The first part of the thesis presents a comprehensive database of chemical and physical properties of nine different varieties of ginger collected from various parts of northeast India. The relative abundance of the mineral contents follows a trend of K > Na > Mg > Ca > Fe > P > Mn > Zn > Cr > Ni > Cu for all the varieties. Ethanol is the best solvent for extracting maximum phenolics (0.96 mg g^{-1}) and flavonoids (1.52 mg g^{-1}) content from dried rhizomes. *Sungro-sung* variety from Nagaland has highest content of 6- gingerol (2.21 wt % dry basis). This is the highest 6-gingerol content found in any ginger variety reported so far. Chemometric tools have clearly segregated *Sungro-sung* followed by *Arunachal Pradesh local* varieties as the best in terms of overall antioxidant as well as nutritive potential. The correlation study within all the properties reveals a strong inverse relationship among the average size with major bioactive constituents of oleoresin and volatile oils of all the varieties.

The second part explores the extraction and purification of antioxidants (ginger oleoresin and volatile oil) from the selected best variety using green solvent. In this context, a single step green process for supercritical CO_2 ($SCCO_2$) extraction coupled with fractionation of dry ginger for simultaneous separation of gingerols rich oleoresin and volatile oil is developed and reported for the first time. The optimization and scale-up validation of this process is achieved in three stages. Firstly, $SCCO_2$ extraction conditions are optimized for the highest yield of mixture extract containing a maximum amount of volatile oil components and major actives [sum of (6+8+10) gingerols and 6-

TH-2386_136107006

shogaol]. The optimum process variables under laboratory scale conditions are: average ginger particle size 253 µm, extraction temperature 40 ºC, pressure 276 bar, flow rate 30 g min^{-1} and 153 min of extraction time. This results in 8.6 % yield of a mixture extract having 37.97 wt % of major actives and 28.3 wt % of volatile oil. In the second stage of experiments, these conditions are scaled up fifty folds on a commercial scale unit. This results in an improvement of 0.1% yield with an increase of 0.34 wt % and 1.2 wt % in the major actives and volatile oil contents, respectively. Finally, the optimum conditions of online $SCCO_2$ fractionation (coupled with optimum extraction conditions) results in 5.95 % oleoresin yield, which is 96.15 % pure and 51.2 wt % of major actives, in a separator operating at 175 bar/40 ˚C. Simultaneously, 2.71 % yield of volatile oil (95.94 % pure) is recovered in another separator operating at 40 bar/40 ºC.

The poor water solubility of the extracted gingerols rich oleoresin severely restricts its application for only lipid-based food products. Hence, the third part of the thesis deals with carrier design to enhance water dispersibility of the extracted oleoresin targeting applications in non-lipid based food products. In this context, a stable formulation of ginger oleoresin for the delivery of ginger actives targeted for food applications involving water based manufacturing processes is developed for the first time. The optimized formulation contains 27 wt % surfactant (Gelucire 44/14), 11 wt % co-surfactant (Transcutol 90), 46.5 wt % of ginger oleoresin, and 15.5 wt % of Capryol 90 as oily vehicle. The average globule size of the dispersion prepared from the formulation is in the nano range (<50 nm) and the dispersion is stable until 24 h. The anhydrous formulation is found to be stable in a long term storage for 90 days in an accelerated condition (40 ºC and 75 % RH). The developed formulation also shows 95.4 % enhancement in water dispersibility of ginger actives over pure oleoresin.

Consequently, the developed formulation is utilized for developing a ginger actives infused fruit based nutraceutical food product. The collated effects of undertaking various process improvement measures in the product development studies result in drastic improvement of the nutritional and functional quality of candied mango slices. Gingerols infusion into mango matrix not only improves the functional quality, but the ginger mango flavor synergy is also responsible for the enhancement in the overall sensory scores of the developed product. Further optimization of the subsequent candying process for identifying equilibrium saturation points for maximum sugar and water loss of mango slices minimizes the loss of natural bioactive compounds present in the mango. Thereafter, the process conditions optimized at bench scale are scaled-up 200

TH-2386_136107006

folds and augmented with commercial-scale vacuum drying at a commercial scale candy manufacturing facility. The manufactured product has 85.6, 76.8, 60.2 % retention in β-carotene, total phenolics, vitamin C, respectively, along with minor color difference and significant improvement in the sensory scores over fresh mango. A strong agreement is achieved in mass transfer properties and all the quality characteristics between bench and manufacturing scale experiments imply excellent scalability and repeatability of the complete process. The current study also results in significant enhancement of vitamin C, TPC, and β-carotene contents by 233.3, 131.8 and 42.7 %, respectively along with superior color, sensory and textural properties, compared with an existing market product. In addition, the drastic reduction of overall process time from 143 to just 30 h is also recorded.

Finally, the effects of gingerols infusion in shelf qualities of the developed candied mango product are investigated. Incorporating nano-encapsulated gingerols into candied mango matrix not only improves the sensory profiles but also significantly retards the browning inducing reactions as quantified by a range of associated quality parameters. This intervention substantially enhances the shelf-life in all conditions of storage. Based on univariate kinetics of all the individual quality parameters, β-carotene is found to be the most abruptly changing parameter. Multivariate analysis of storage data is a more holistic approach as it considers the cumulative effect of deteriorations caused by multiple spoilage factors. Highest predicted shelf life of gingerols infused candied mango product is 338 days as against 155 days for the non-infused product when product is stored in MET pouch at 25 ^{o}C. Slower deterioration of all quality parameters is observed in MET than EVOH packaging material for both products in all temperatures. In order to verify the preciseness of calculated predictions, the actual shelf life of control samples (non-gingerols infused) stored in EVOH pouches at 25 ^{o}C are compared with predicted values. Chemometrics based 144 days of predicted shelf life is close to the actual shelf life of 142 days but varies significantly from 185 days of univariate kinetics based predictions.

TH-2386_136107006

TH-2386_136107006

Chapter 1

Introduction and Review of Literature

This chapter briefly gives fundamental information about ginger, its chemistry and medicinal properties. It reviews various literature in the area of quality and characterization of different ginger varieties found around the world. Following this, various techniques and process parameters involved in the processing and extraction of antioxidants from ginger are presented. State of art on processing of ginger extract, its application in food product development as well as shelf life studies are also discussed. The review of literature follows to the research gaps and finally specific objectives and organization of thesis are presented.

TH-2386_136107006

TH-2386_136107006

1.1 Background

Antioxidants play a vital role in preventing or delaying autoxidation and have attracted commercial interest as additives and stabilizers in food product development, dietary supplements, natural health products, and a range of pharmaceutical products. The oxidative deterioration in food products is responsible for a range of spoilage indicators like color deterioration, off flavors, loss of texture and product integrity (Taoukis et al., 1997). These factors ultimately lead to decrease in nutritional profile of products and safety value due to formation of secondary compounds which are potentially toxic. In order to address these problems, the food industries employ synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), tertiary-butyl hydroquinone (TBHQ), propyl paraben, and sodium benzoate (Sehwag et al., 2018). In addition, synthetically produced flavors and functional ingredients like vitamins, minerals, essential fatty acids etc. are also used to impart desired nutraceutical qualities in the processed food products.

A stream of recent literature has established the carcinogenic effects of long term use of synthetic antioxidants on human health (Sindhi et al., 2013). This, along with increasing interests of consumers for natural/herbal-based products, has initiated action for strict regulatory measures leading to even prohibition of some class of synthetic antioxidants by many countries like USA, Canada, Japan, most countries of Europe etc. This has also paved the way for research in the development of new generation minimally processed food products employing natural antioxidants, flavors, additives, isolated from herbs and spices.

Consequently, the market demand for food products manufactured with clean labels and green additives having multifunctional and tailored nutraceutical properties with enhanced shelf lives is increasing exponentially (Vieira da Silva et al., 2016).

1.2 Plant based natural antioxidants

Herbs and spices are a reservoir of natural chemical agents having therapeutic properties (Parthasarathy et al., 2008). Plants have been a vital source of food as well as medicine for humans since time eternal. In addition, herbs and spices are used for their unique flavor and preservative effects in culinary avenues. Plants produce a host of phytochemicals for their survivability, defence mechanism, maintenance, and as

attractants for pollinators. Plants also produce these substances to protect themselves against UV light for their survival and for adaptation to their environment (Yeung et al., 2018). Most of these compounds have been clearly proven to show a high antioxidant activity (Parthasarathy et al., 2008). Because of this, plants have been an integral part of traditional medicine practices of different regions of the world as well as in some of the most revered and well established medicine systems like Ayurveda (Hazarika and Kakoti, 2013). However, in the recent years, modern science has started paying attention to such natural compounds.

Table 1.1: Natural antioxidants found in plants [adapted from Parthasarathy et al. (2008)]

Herbs name	Antioxidants
Clove	Phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol), tannins
Ginger	Shogoal, gingerol
Mace	Myristphenone
Marjoram	Beta-carotene, beta-sitosterol, caffeic-acid, carvacrol, eugenol, hydroquinone, linalyl-acetate, myrcene, rosmarinic-acid, terpinen-4-ol
Nutmeg	Myristphenone, phenolic volatile oils, phenolic acid (caffeic acid), flavanols (catechin)
Oregano	Caffeic acid, p-coumaric acid, rosmarinic acid, caffeoyl derivatives, cavacrol, flavonoids
Red pepper	Beta-carotene, beta-sitosterol, caffeic acid, campesterol, camphene, capsaicin, capsanthin, chlorogenic acid, eugenol, gamma terpinene, hesperidin, myristic acid
Rosemary	Carnosol, 12-O-methylcarnosic, rosmanol, caffeic acid, rosmarinic acid, caffeoyl derivatives, phenolic diterpenes (carnosic acid), carnosol, epirosmanol, flavonoids
Sage	Rosmanol, epirosmanol, phenolic acids (rosmarinic acid), phenolic diterpenes (carnosic acid), flavonoids
Sesame seed	Sesaminol, α-tocopherol, sesamol
Turmeric	Curcumin, 4-hydroxycinnamoylmethane
Thyme	Phenolic acids (gallic acid, caffeic acid, rosmarinic acid), thymol, phenolic diterpenes, flavonoids

Phytochemicals are a large group of bioactive antioxidants derived from plants which have potential protective effects against diseases which consists of flavonoids, phenolic compounds, carotenoids, plant sterols, glucosinolates and other sulphur-

TH-2388_132107006

containing compounds **(Table 1.1).** Spices and herbs are great sources of natural antioxidants. In addition to their health beneficiating properties, they are readily assimilated by the body.

Out of all the natural antioxidants enumerated, gingerols and shogaols have been very well established for their therapeutic potential in a very wide range of ailments (Semwal et al., 2015). Ginger also happens to be the oldest spice used for a range of medicinal properties in all the forms of traditional medicines practiced around the world (Shukla and Singh, 2007).

1.3 Ginger

Ginger is known for its characteristic flavor and health benefits ever since a very long time and is one of the most widely used spices. India has been historically known for spices and ginger finds importance in popular classical Sanskrit texts like *Ashtanga-hridayam, Sushrutha samhita and Charaka samhita*. It is a key ingredient in traditional medical practices of all the ginger growing countries. In Ayurveda, it is called *Mahaoushadha* and has been recommended to cure numerous ailments including common cold, influenza, cough, arthritis, rheumatic disorders, migraines, headaches, cardiac palpitations and hypertension (Shukla and Singh, 2007).

1.3.1 Significance of ginger in food product development

Among all spices, ginger (*Zingiber officinale*) is one of the most prominent utilities used in the food processing industries throughout the world. Its fresh form is mostly used in the manufacturing of preserved ginger (with brine or sugar syrup). The paste serves as “base” material for industrial production of a variety of food products. The juice is also used in a few specialty applications. Dried ginger is the most important processed form and constitutes almost 70 % of the trade globally (Govindarajan and Connell, 1983). It is used directly as a spice and in the production of extracts (ginger oil, oleoresin, and variety of other products) owing to its rich contents of bioactive compounds. The preferred palettes of characteristic parameters vary according to the desired functional attributes in the end product.

The oleoresin (non-volatile extract) contains pungent principles like 6, 8 and 10-gingerols as major active ingredients, responsible for the characteristic hot taste. These have been extensively studied for their wide range of health benefiting qualities. Owing

to the distinct pungency of gingerols, the oleoresin of ginger is widely utilized as a commercial flavoring agent. A range of commercial products are manufactured to exploit the rich flavor synergy resulting from the unique pungent flavor of oleoresin. Some of the popular ginger flavored market products include bakery, ice-cream, confectionary, range of health products, milk-based products etc. Its incorporation not only synergistically improves the unique flavor palette of the developed food product and enhance its functional profile, but the actives present also results in enhancement of shelf stability by delaying the autoxidative changes. Hence, ginger oleoresin exhibits tremendous commercial potential for application as a single, green, multifunctional, natural food flavoring, preservative and nutraceutical agent.

1.3.2 Chemistry of ginger

Ginger is rich source of phytochemicals which have been demonstrated to be highly beneficial for health. The ginger rhizome contains steam volatile oil, fixed fatty oil, pungent compounds, resins, proteins, cellulose, pentosans, starch and mineral elements (Govindarajan and Connell, 1983). The composition of these components varies with type of cultivar, region, agroclimatic conditions, maturity and nature of rhizome (Parthasarathy et al., 2008). Primarily, ginger is composed of a number of pungent compounds, volatile oils, carbohydrates, fats, proteins, vitamins and trace elements. Out of these, the important ones are gingerols, shogaols and zingerons which contribute to pungency.

Ginger also contains up to 4 % of fragrant essential oil (on fresh weight basis), which mainly consist of a high content of sesquiterpene hydrocarbons in particular (-)-Zingiberene (35 %), *ar*-curcumene (18 %) and farnesene (10 %), with lesser amounts of bisabolene and *β*-sesquiphellandrene. A smaller percentage of at least 40 different monoterpenoid hydrocarbons are also present with 1, 8-cineole, linalool, borneol, neral, and geraniol being the most abundant (Govindarajan and Connell, 1983). The characteristic organoleptic properties of ginger are due to steam volatile oil and non-volatile solvent extractable pungent components. The pleasant aroma of ginger is caused by more than 70 constituents present in the steam volatile oil. Among these, sesquiterpene hydrocarbon, (-)-Zingiberene predominate and account for 20 – 30 % of the oil obtained from dry ginger (Parthasarathy et al., 2008). Many of these volatile oil constituents contribute to the distinct aroma of ginger. This non-volatile oil of ginger rhizome (also known as ginger oleoresin) contains biologically active constituents such

TH-2388_134107006

as gingerols, shogaols, paradols and zingerone, which produce a hot pungent sensation in the mouth. The gingerols, are identified as the major active components in the fresh rhizome (Govindarajan and Connell, 1983). In addition, the shogaols are thought to be the dehydration products of the gingerols, derived from thermal processing (drying/heating) or long-term storage (Bhattarai et al., 2001) and are more pungent than the gingerols (Zancan et al., 2002). These actives are phenolic ketones which exist as a series of homologues (4, 6, 8, and 10-gingerols and shogaols) with a range of unbranched alkyl chain lengths (Bhattarai et al., 2001). Out of these, 6, 8 and 10 gingerols are the major active components and are thermally labile compounds which on exposure to heat, degrade to shogaols and Zingerone through reverse aldol reaction. Out of all, 6-gingerol is reportedly the most abundant constituent in the gingerols series. The warm pungent characteristic taste of ginger is caused by a number of components predominated by gingerols followed by shogaols and zingerone (Govindarajan and Connell, 1983). Since gingerols and shogaols are enantiospecific, **Figs. 1.1-1.3** shows their general chemical structure along with Zingerone.

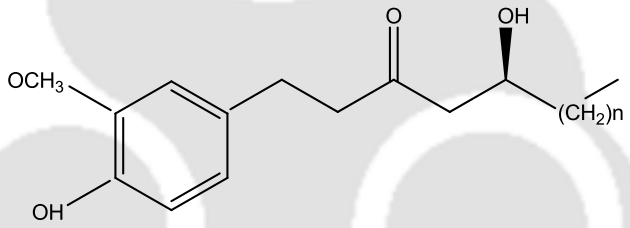


Fig. 1.1: General structure of Gingerol, where n=2, 4, 6, 8

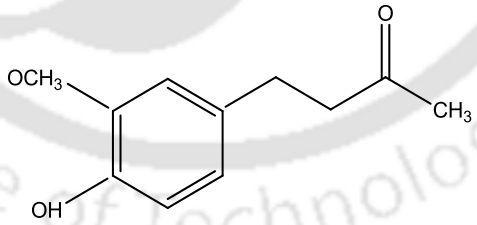


Fig. 1.2: Structure of zingerone

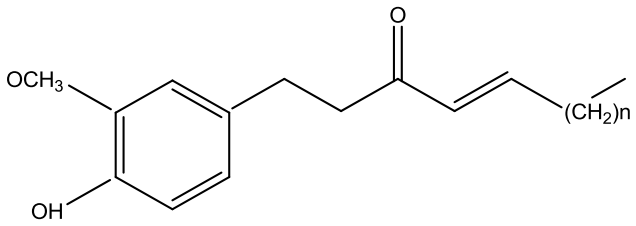


Fig. 1.3: General structure of Shogaol, where n=2, 4, 6, 8

1.3.3 Medicinal properties

The medicinal properties of ginger are attributed to its spicy, pungent constituents, mainly gingerols, which stimulate thermoregulatory receptors (Wang et al., 2018). Gingerols and shoagols possess a wide range of pharmacological and physiological effects, which include cardiovascular, gastro-intestinal (antiemetic, antinausea, antiulcer), antioxidant, anti-inflammatory, antimicrobial (analgesic, sedative, antipyretic, antibacterial), as well as thermogenic activities (Srinivasan, 2017). At high concentrations, gingerols inhibit lipid peroxidation in rat liver microsomes (Xu et al., 2016). Gingerols have shown to enhance platelet function due to inhibition of thromboxane hormone formation (Srinivasan, 2017). Ginger has also been suggested to reduce the inflammation processes (Bakht et al., 2014). Furthermore, ginger acts as a hypolipidemic agent in cholesterol-fed rabbits (Ogino et al., 2018). Its major pungent constituent, 6-gingerol has been reported to exhibit antioxidant activity against linoleic acid. It also retards peroxidation of phospholipid liposomes and scavenges activity of free radicals like trichloromethyl peroxyl- and 2, 2- diphenyl-1-picrylhydrazyl (DPPH) (Srinivasan, 2017; Wang et al., 2018). In addition to these antioxidative effects, a study revealed that 6-gingerol inhibits nitric oxide synthesis in activated J774.1 macrophages and prevents oxidation and nitration reactions induced by peroxynitrite, a strong reactive nitrogen species (Ippoushi et al., 2003).

Ginger has been shown to be effective against tumor growth, rheumatism and migraine and is active as an antioxidant (Shukla and Singh, 2007), hence ginger is utilized as an ingredient in various commercial natural products in emerging nutraceuticals and functional foods (Kubra and Rao, 2012). Recently, use of ginger has been increased significantly because of its low toxicity and broad spectrum of biological and pharmacological applications (Shukla and Singh, 2007).

1.3.4 Applications

Ginger is commonly used in fresh, preserved and dried forms in different culinary cultures around the world. Commercially, it is most widely traded in dried form. This form is used industrially to prepare extractives which are used as flavorings for food, beverage and in pharmaceutical applications.

The application and uses of ginger can be broadly classified as under:

(a) Fresh ginger

- Direct consumption in raw form.

TH-2388_136107006

- Preparation of preserved ginger in brine or sugar solution.

(b) Dry ginger

- Directly used as an ingredient in culinary purposes.
- Used as spice/condiment in powdered/non-powdered form.
- Used in Ayurveda as well as in a variety of folk/local medicine forms of the world.
- Industrially used for extraction of oleoresin, volatile oil, flavors etc.
- Utilized to manufacture a range of active pharmaceutical ingredients (APIs).
- Utilized in development of newer and improved varieties of ginger/ genetic engineering research.
- Powder used in preparation of a variety of processed bakery, confectionary and beverage products.
- Ginger volatile oil is used as a food flavourant in the manufacturing of a variety of beverages.
- Oleoresin is used as a flavourant in a variety of milk and lipid-based food products.

1.3.5 Quality of ginger varieties found around the world

Depending upon the geographical factors, the constituents of desired parameters may vary widely in different ginger varieties of the world. Therefore, screening of ginger varieties is important to serve as a guideline to foster applied research in high-end product development and to enhance economic margins. Because of the complexity of the compositional variation in different ginger types, it is often difficult to compare samples from different agro-climatic zones. **Table 1.2** gives an account of the work carried out by various researchers on the characterization of different ginger varieties of the world. Most of the published works on ginger characterization concentrate on antioxidant properties (Semwal et al., 2015; Yeh et al., 2014; Yudthavorasit et al., 2014) whereas a very few have studied other parameters like minerals (Pandotra et al., 2015) and biochemical properties (Pattnaik et al., 2016).

While screening a ginger variety for commercial applications, the preferred parameters vary according to the desired attributes in the end product. For example, fresh ginger is expected to have constituents such as volatile oil, fiber, moisture content, total soluble solids etc. at limits optimum for industrial process, ideal to a specific consumer

product. Similarly, dried ginger should have maximum quality characteristics in terms of volatile oil, oleoresin, gingerols along with optimal contents of fiber and minerals (Ismail and Lingamallu, 2012).

Table 1.2: Work carried out by various researchers on the characterization of different ginger varieties

No. of Varieties	Origin	Parameters considered	References
18	India	Total phenolics, gingerols, and total antioxidant activity	(Sanwal et al., 2010)
2	Taiwan	Proximate analysis, antioxidant activities of various solvent extractives, gingerols, shogaols and essential oil profiling	(Yeh et al., 2014)
5	India, China, Malaysia, Vietnam, and Thailand	9 major active compounds	(Yudthavorasit et al., 2014)
10	Northeast India	Oleoresin yield, 6 gingerol content, essential oil yield and its composition	(Kiran et al., 2013)
1	South India	Proximate composition, solvent extractives, and antioxidant activity	(Adel and Prakash, 2010)
17	Australia	Essential Oil content and composition	(Wohlmuth et al., 2006)
20	East and South India	Biochemical properties - protein, phenol polyphenol oxidase and fiber content	(Pattnaik et al., 2016)
1	Nigeria	Proximate composition and mineral profile	(Latona et al., 2012)

TH-2388_138107006

1.3.5.1 Ginger endemic to northeast India

Northeast region of India is considered to be one of the richest biodiversity hotspots, home to innumerable commercially important as well as rare plant species. This region is treasured for one of the best qualities of fruits and vegetables which are considered among the most important varieties the world over. Ginger is the main cash crop here and this region accounts for over 70 % of total production of ginger in India, making the country largest producer in the world (Majumder et al., 2011; Rahman et al., 2009).

1.4 Extraction and purification of antioxidants from ginger

Commercially, dried ginger is most widely utilized for the production of oleoresin and volatile oil. Hence, the cost of traded ginger is directly based on oleoresin, volatile oil and content of major actives. Conventional techniques of solvent extraction and steam distillation are the most common methods for commercial production of ginger oleoresin and volatile oil.

These are however associated with major drawbacks such as complex process involving multiple unit operations (extraction, separation etc.), and high operating temperatures which cause degradative changes to major active compounds as well as total extract yields. Apart from this, the use of organic solvents (hexane, acetone, dichloromethane, trichloromethane, isopropanol, ethanol, ethylene dichloride etc.) has been reported to be hazardous on health while some solvents have shown carcinogenic effects (Mukherjee et al., 2014). The growing health concerns in recent times have thus made GRAS (Generally Recognized as Safe) solvents very popular for commercial extraction of natural bioactive compounds.

Supercritical CO_2 ($SCCO_2$) is a promising solvent owing to its low critical pressure of 73.82 bar and temperature of 31 ^oC. Heat sensitive compounds can be extracted without any degradation and it is an environmentally acceptable solvent which does not introduce any traces of harmful chemicals (Pradhan et al., 2010; Reverchon, 1997). In addition, $SCCO_2$ is non-toxic, non-explosive, abundantly accessible in high purity, and is easily removed from the extracted material instantly without leaving any residue. The solvating capacity of $SCCO_2$ can be easily adjusted by slightly altering its temperature and pressure for selective extraction of antioxidants, pigments, flavors, fatty

acids, essential oils etc. from plant matrix (Naik et al., 1989; Rout et al., 2009; Rout et al., 2008).

Hence, it becomes an imperative to explore $SCCO_2$ for extracting highest quality ginger extract, having clean label. Various researchers have produced ginger extract using $SCCO_2$ technology. Said et al. (2015) have reported ginger oleoresin yield of 6.9 % at 35 ^oC, 250 bar pressure and 15 g min^{-1} $SCCO_2$ flow rate. Chen et al. (2011) have reported optimum process conditions to be 60 ^oC, 275.8 bar, particle size less than 595 μm, at 90 min extraction time, giving a maximum ginger volatile oil yield of 2.7%. Badalyan et al. (1998) attempted to understand the composition of extract separated from dry ginger in a wide range of process parameters, namely: temperature 9 – 35 ^oC, pressure 60 – 100 bar, flow rate of $SCCO_2$ 0.2 – 0.5 kg m^{-2} s^{-1}. They found that the separated extract was a mixture of essential oil and oleoresin and their proportion in the extract was highly dependent on process parameters chosen. Lower range of parameters resulted in higher contents of essential oil whereas, oleoresin content dominated the extract at higher end of chosen process variable range.

Process scale-up studies give critical insights which help in making important choices like feasibility comparison with existing conventional techniques and ultimately in the commercialization of newer state of the art. Recently, Salea et al. (2017) have optimized ginger extraction at a laboratory scale $SCCO_2$ facility and reported the highest yield of 3.1 % (primarily volatile oil with some percentage of oleoresin) with the 6-gingerol content of 20.7 %. In their study, the reported process conditions were 150 bar, 35 ^oC, and 15 g min^{-1}, which were scaled-up to fifty times at a commercial facility resulting in an increased yield of 3.8 % and decreased 6-gingerol content of 18 %. A summary of research findings on extraction work on ginger has been presented in **Table 1.3**.

1.5 Tailoring ginger oleoresin for food applications

Despite the attractive portfolio, the lipid nature of ginger oleoresin limits its applications to only oil-based products. Besides, the application in food supplements and pharmaceutical sector, it is also highly limited due to poor water solubility of its actives which severely reduces its oral bioavailability and hence efficacy (Xu et al., 2016). Moreover, gingerols are highly susceptible to temperature and get dehydrated into shogaols, paradols and zingerone (Bhattarai et al., 2001).

TH-2388_136107006

Table 1.3: Summary of published literature on $SCCO_2$ extraction of ginger

Variety	Process details	Equipment details	Optimum process parameters	Product portfolio			References
				Type	Yield %	Extract quality parameters	
Laboratory study							
Varanasi local (India)	Ultrasound assisted $SCCO_2$	1 extractor (0.5 L) and 1 separator (0.5 L)	Temp: 35 °C, ρ_{avg}: 250 µm, Pressure: 250 bar, Q_{CO2}: 15 g min^{-1}, Time: 60 min	Mixture	8.2	N.A.	(Said et al., 2015)
Wonogiri (Indonesia)	$SCCO_2$ extraction	2 extractor (2 L each) and 1 separator (N.A.)	Temp: 35 °C, ρ < 600 µm, Pressure: 150 bar, Q_{CO2}: 15 g min^{-1}, Time: 160 min	Mixture	3.1	20.7 wt % 6-gingerol 6-gingerol: 0.6 % (on dry wt basis)	(Salea et al., 2017)
Cochin (India)	$SCCO_2$ extraction	1 extractor (0.75 L) and 2 separator (0.1 L each)	Temp:39.85 °C, ρ <600 µm, Pressure: 195 bar, Q_{CO2}: 15 g min^{-1}, Time: N.A.	Oleoresin	4	18.5 wt % 6-gingerol 6-gingerol: 0.7 % (on dry wt basis)	(Yonei et al., 1995)
Pingtun local (Taiwan)	$SCCO_2$ extraction	1 extractor (0.1 L)	Temp: 60 °C, ρ:< 595 µm, Pressure: 275.8 bar, Q_{CO2}: N.A., Time: 90 min	Volatile oil	2.7	-	(Chen et al., 2011)
Queensland (Australia)	$SCCO_2$ extraction	N.A.	$SCCO_2$ density between 0.7 – 0.9 g mL^{-1}	Volatile oil	N.A.	25.9 wt % α - zingiberene[a]	(Bartley and Foley, 1994)
Meghalaya (India)	Steam Distillation	Clevenger's Type	N.A.	Volatile oil	3.2	100 % pure volatile oil with 29.9 wt % α - zingiberene[a]	(Ravi Kiran et al., 2013)
Cassumunar (Thailand)	Solvent-free microwave extraction (SFME)	Microwave equipped with Clevenger's type apparatus	Microwave power: 700 W, extraction time: 70 min	Volatile oil	1.1	100 % pure volatile oil with 0.02 wt % α - zingiberene[a]	(Yingngam and Brantner, 2018)
Tongling White (China)	Ionic liquid based ultrasonic assisted extraction (ILUAE)	Ultrasonic water bath	[C4mim]BF4 conc. :1.5 M, Ultrasound power: 200 W, temp: 25 °C, solid/liquid ratio: 1:20 (g mL^{-1}), time:10 min	Mixture	N.A.	6-gingerol: 1 % (on dry wt basis)	(Kou et al., 2018)
China	$SCCO_2$ extraction followed by purification with macroporous resin	N.A.	Temp: 40.9 °C, Pressure: 225.9 bar, Time: 265 min	Volatile oil	1.2	46.6 wt % pure volatile oil	(Lei et al., 2016)
Scale-up study							
Wonogiri (Indonesia)	$SCCO_2$	2 extractor (50 L each) and 1 separator (N.A.)	Temp: 40 °C, ρ_{avg}: 250 µm, Pressure: 276 bar, Q_{CO2}: 30 g min^{-1}, Time: 144 min	Mixture	3.8	18 % 6-gingerol 6-gingerol: 0.7 % (on dry wt basis)	(Salea et al., 2017)

a: considering α – zingiberene as a major volatile ingredient for comparison purpose; $SCCO_2$: Supercritical carbon dioxide; Temp: temperature; ρ_{avg}: average ginger powder particle size, Q_{CO2}:$SCCO_2$ flow rate

These drawbacks can be efficiently addressed by various state of the art encapsulation and solubilization technologies. In addition, the available tools can also improve the therapeutic potential of ginger actives thereby expanding its application spectrum in food product development involving water-based manufacturing processes/applications.

Several solubilization technologies have been developed, including nanoparticles, complexion, micelles, emulsions, solid dispersion, etc. (Rezaei et al., 2019). The self-micro emulsifying drug delivery systems (SMEDDS) approach could be one of the most powerful techniques for enhancing solubility of sparingly soluble actives (Pouton, 2006). SMEDDS is basically an isotropic mixture of actives/drug/natural extract, carrier oil, surfactant, and co-surfactant which can form fine oil-in-water micro/nanoemulsion when introduced into aqueous phases under gentle agitation (Pouton, 1997). The improvement in the wettability and dispersibility of the actives/drug with high lipophilicity enables enhancement in oral absorption of the active compound in SMEDDS formulations. The relative ease of spontaneously forming dispersion in water for designing SMEDDS based formulations could be a promising method for delivery of ginger actives without the use of sophisticated apparatus.

Moreover, the encapsulation of ginger actives by the carrier polymers and relatively inert interactions between them could enhance the physicochemical stability of ginger actives ultimately leading to high shelf-life of the formulation (Date and Nagarsenker, 2007). The ginger oleoresin could be homogeneously encapsulated in carrier polymers, and the relative ease of filling the solid/semi-solid formulation in hard/soft gel capsules along with excellent scalability and economic viability makes this technology commercially attractive (Kuentz, 2011).

Also, the application of carriers with bio enhancing properties could further boost the quality and efficacy of designed formulation for ginger actives. Gelucire 44/14 is one such commercially available semi-solid amphiphilic excipient with a high hydrophilic-lipophilic balance (HLB) value of 14 and low melting point of 44 ^{o}C. It comprises 20 wt % of C8 to C18 mono-, di- and triglycerides, 72 wt % of C8 to C18 mono- and di-fatty acid esters of polyethylene glycol (PEG)-1500 and 8% free PEG-1500 (Panigrahi et al., 2018). When a formulation of actives encapsulated in Gelucire 44/14 comes in contact with gastrointestinal fluids, it spontaneously forms micro emulsion leading to enhanced solubilization as well as increase in the bioavailability of the actives (Chambin and Jannin, 2005).

TH-2388_13107006

TH-2386_136107006

Table 1.4: Summary of important published reports on encapsulation and solubilization techniques ginger oleoresin formulations

Sr. No.	Ginger material used	Formulation portfolio	Formulation characteristics	Achievements	Reference
1	Solvent extracted and purified 6-shogaol extract with 98.7 wt % purity	Method: Solid Lipid Nanoparticles Excipients: Medium-chain triglycerides, glyceryl monostearate, tween 80 and span 80	Particle size: 73.6 nm, Zeta potential: -15.2 mV,	Actives loading: 5.9 wt % Enhancement of (a) 29 % solubility and (b) anti-gout activity	(Wang et al., 2018)
2	Solvent extracted and purified 6-gingerol extract with 75.3 wt % purity	Method: Solid Lipid Nanoparticles Excipients: Ethyl oleate, Cremophor EL35 and 1,2-Propanediol	Particle size: 73 nm, Zeta potential: -2.5 mV,	Actives loading: 6.4 wt % Enhancement of (a) 70 % solubility and (b) oral bioavailability	(Xu et al., 2016)
3	$SCCO_2$ extracted ginger oleoresin with 30 wt % of actives [(6+8) gingerols and 6-shogaol]	Method: Solid Lipid Nanoparticles Excipients: Medium-chain triglycerides, Lysolecithin, Glycerin and hydroxypropyl methylcellulose	Particle size: 114 nm, Zeta potential: N.A.	Actives loading: 1.5 wt % Enhancement of (a) 3 folds solubility and (b) pharmacokinetic behavior	(Ogino et al., 2018)
4	HPLC grade 6-gingerol with 97 wt % purity	Method: Phytosome complexed with chitosan Excipients: Soya lecithin and chitosan	Particle size: 254 nm, Zeta potential: -13.1 mV	Actives loading: 7.3 wt % Enhancement of anti-bacterial activity	(Singh et al., 2018)
5	Ginger oleoresin with 0.1 wt % of actives (6-gingerol + 6-shogaol)	Method: Spray chilled solid lipid microparticles Excipients: Palmitic acid, oleic acid or palm fat	Particle size: 85.3 µm, Zeta potential: N.A.	Actives loading: 1.4 wt % N.A.	(Oriani et al., 2016)

The unique composition and physicochemical properties make it an ideal candidate for designing carrier systems for poorly water-soluble and temperature-sensitive active ingredients. Gelucire 44/14 has been reported to improve solubility, bioavailability, dissolution rates, intestinal permeability both in-vitro and in-vivo for a wide range of active pharmaceutical ingredients. It has also been equally effective for phytochemicals like vitamin E (Barker et al., 2003), curcumene (Joshi et al., 2013), quercetin (Jain et al., 2013), lycopene (Faisal et al., 2013), *Brucea javanica* oil (Huang et al., 2018).

The emulsification efficiency of Gelucire 44/14 could be furthermore improved by using it in combination with other surfactants, co-surfactants, and oily vehicles. Some of the reported excipients used in combination with Gelucire 44/14 are Tween 80, Transcutol-HP, various variants of Kolliphor RH, PEG, PG, Capryol 90, Lauroglycol 90, etc. which have resulted in efficient and thermodynamically stable SMEDDS with dispersions in the nano (particle size <100nm) range (Shakeel et al., 2013). **Table 1.4** summarizes the important results of various solubilization technologies.

1.6 Ginger based food product development

The choice of natural antioxidants to be fortified in a specific food matrix is often dictated by consumer preferences. Some commercially exploited tailored attributes are (a) food colorings resulting from natural anthocyanins and carotenoids, (b) antimicrobial effects of plant essential oils, antioxidant and (c) shelf-life enhancement quality of various kinds of plant extracts. Moreover, a range of health-specific qualities like anticancer, anti-diabetic, memory enhancer, high fiber, low glycemic index of natural extracts, have also been imparted in model food systems by using specific natural antioxidants having desired qualities (Yeung et al., 2018). For development of new generation minimally processed food products, it is often challenging to impart multiple quality attributes in a single food product.

Among the various available directions towards incorporating natural antioxidants into food matrix, the osmotic-assisted candying process is one of the simplest, scalable and most effective means of infusing desired functional ingredients into fruits and vegetable matrix. In addition to new product development, it also results in value addition and substantial improvement in the shelf life of fresh fruits and vegetables.

TH-2388_136107006

The low moisture content and improved sensory profiles of the candied fruit product make it a popular stand-alone item. Candied fruits production process essentially involves saturating the fruit pieces with sugar successively in increased strength of sugar solutions. Mechanistically, three kinds of counter diffusional mass transport phenomena take place simultaneously during the process. Firstly, osmotic dehydration (OD) or dewatering of fruit samples takes place. The osmotic pressure difference between the fluids of the natural matrix and the surrounding sugar solution imparts driving force for OD. Secondly, the concentration gradient drives the impregnation of sugar from the osmotic solution into fruit matrix. The third kind of transport involves leaching of fruit's natural solutes like vitamin C, minerals etc. into the sugar solution along with water during OD (Khan et al., 2008; Rastogi et al., 2002), thus leading to loss of nutritive value of the final product. Process conditions like concentration, temperature, and type of osmotic agent (sugar/salt), shape, size and morphology of the fruit, and the ratio of fruit to osmotic solution play an important role in determining the rate of the overall osmotic process as well as on the final product quality (Ranganna, 1986; Rastogi et al., 2002).

Some promising works involving candying process for infusing desired natural antioxidants in development of fruit candies with tailored nutraceutical properties are, anthocyanin in watermelon rinds (Bellary et al., 2016), and grape phenolics (Cappa et al., 2015). Infusion of non-water-soluble actives like curcuminoids in banana (Bellary and Rastogi, 2014) and coconut slices (Bellary et al., 2011), inulin and piquin-pepper oleoresin in mango slices (Jiménez-Hernández et al., 2016) have also been reported. In addition to imparting desired color such as red and yellow from anthocyanins and curcuminoids, respectively, infusate imparted desired flavor in case of inulin and piquin-pepper oleoresin. In general, a simultaneous overall improvement in storage stability was also observed for all the developed candied fruits. Summary of best literature has been presented in **Table 1.5**.

Mango, king of all the fruits, is popular for its unique taste, aroma and high contents of β-carotene and vitamin C. Owing to its distinct flavor profile it finds its way into a wide range of culinary as well as processed food applications across the globe. The low moisture content and improved sensory profiles of its candied form make it a popular stand-alone item. It also finds extensive application in ice cream, dairy, bakery, and confectionary industries (Giraldo et al., 2003). The incorporation of pungent ginger actives in candied mango has potential to synergistically enhance the unique sweet-sour flavor palette of mango along with improving its nutraceutical properties.

TH-2388_11607006

Table 1.5: Summary of important published reports on infusion of natural antioxidants for food product development

Sr. No.	Cultivar of mango used	Infusate	Details of optimum process parameters			Final product quality characteristics	Reference
			Infusion	Candying/OD	Drying		
1	*Creole* (Mexico)	Inulin + pequin-pepper oleoresin	Combined OD + infusion Osmotic solution of 60 wt % (25 % infusate + 45 % sucrose) Temperature: 40 °C Treatment time: 120 min Fruit/solution ratio: 1/30		Oven drier Temperature: 80 °C Time: 24 h	Vitamin C: 54 %[a] β-carotene: 95.8 %[a] Total Phenols: 79.9 %[a] Total oil: 2.2 %[a] a_w=0.5	(Jiménez-Hernández et al., 2016)
2	*Tommy Atkins* (Sweden)	Calcium ($CaCl_2$)	Combined OD + infusion Sugar solution of 45 °Brix + 1 wt % infusate Temperature: 25 °C Treatment time: 15 h Fruit/solution ratio: 1/10		Hot air drier Air vel.: 0.2 m s^{-1} Temperature: 70 °C Time: 2 h	Vitamin C: 60 %[a] β-carotene: 76.9 %[a] a_w=0.6 Moisture content: 16 %[b]	(Guiamba et al., 2016)
3	*Alphonso* (India)	Aloe Vera gel	Combined OD + infusion Sugar solution of 45 °Brix + 5 wt % infusate Temperature: 45 °C Treatment time: 60 min Fruit/solution ratio: 1/20		Vacuum drier Vacuum: 0.5 Pa Temperature: 45 °C Time: 2 h	Vitamin C: 36 %[a] Total Phenols: 51.5 %[a] Moisture content: 1.5 %[b]	(Chakraborty and Samanta, 2015)

a: % retention with respect to values of fresh mango on a dry weight basis, b: expressed on a dry weight basis, a_w: water activity

The existing industrial process for candied mango production involves preliminary processing steps like washing the fruit, peeling, destoning, pricking the useful flesh, steam blanching, and cutting into desired shapes. This is followed by subjecting the treated slices to candying process, for slow impregnation of sugar by holding them for 24 h or more (at normal room temperature) in successively increasing strengths of sugar syrup from 30 to 70 ^{o}Brix till the slices are saturated with sugar. After osmotic treatment, the candied slices are dried to a final moisture content of 12 - 14 % having a water activity of 0.4 - 0.5 (Ranganna, 1986). A thin layer of sugar/dextrose is applied to the slices in the dehumidified environment, and the product is finally packed in suitable containers as shown in flow chart, **Fig. 1.4**.

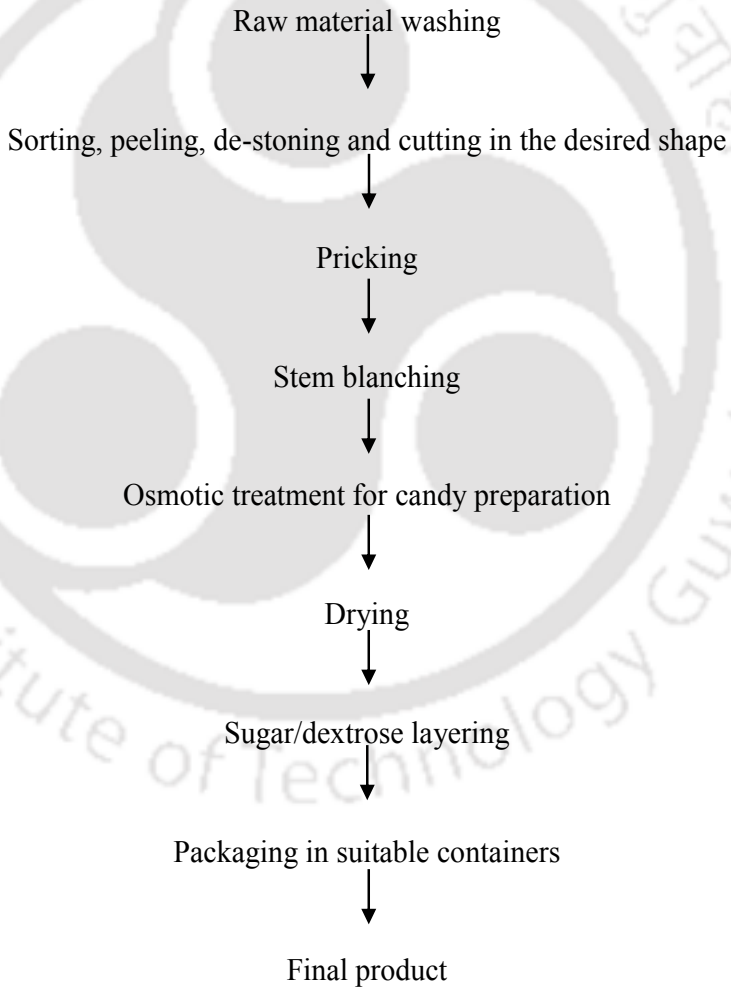


Fig. 1.4: Industrial process of mango candy production

1.7 Shelf-life/storage studies

Among various reasons for deteriorative changes during product storage, non-enzymatic browning has been reported to be one of the major causes for fruit/vegetable based candied/osmotic dehydrated (OD) products. The literature suggests that the degradation kinetics of vitamin C and β-carotene to be most closely associated with kinetics of increase in non-enzymatic browning among reported storage behavior of various fruits (Wibowo et al., 2018; Wibowo et al., 2015). Considering the complexity of the food matrix, the effects of other factors inducing
spoilage reactions occurring simultaneously cannot be ruled out. A systematic study of a range of quality parameters which are critical to color change and their interactive effects involving other important antioxidant compounds could be critical for explaining the browning phenomena.

Shelf-life prediction of industrial candied products is often done in accelerated conditions. This is a difficult task as it involves a critical study of how the quality determining attributes deteriorate over a period of short time under elevated temperatures. It becomes even more challenging when a single accurate prediction has to be made on the basis of different cut-off criteria resulting from multiple critical attributes and their associated kinetic parameters. Chemometrics based multivariate analysis tools like principal component analysis (PCA) is a data reduction tool which extracts single principal components (PCs) from multivariable shelf life data. This simplifies the kinetic interpretations by giving single cut-off criteria, which encompass the cumulative effects of all critical parameters as well as their interaction effects. This could ultimately result in an accurate shelf-life predictions (Wibowo et al., 2018). Incorporation/infusion of natural antioxidants as a means for the sole aim of enhancing shelf-qualities of various food systems has been explored by various researchers. **Table 1.6** summarizes important results of shelf-life extension in a range of model food systems when fortified with plant-based natural antioxidants.

1.8 Specific knowledge gaps

1.8.1 Ginger varieties

Considering the importance of northeast region of India as one of the richest biodiversity hotspots in the world, it is surprising that there are only few reports exploring

TH-2388_1311007006

Table 1.6: Summary of reports on the enhancement of shelf-quality of food products using plant extracts by various researchers

Plant material used	Food matrix	Purpose	Optimum dose	Result	Reference
Essential oil of clove bud (CBEO) and curry leaf (CLEO)	Burfi	Improvement of antioxidant (AOX) and antimicrobial (AM) potential	0.1 ppm CBEO and 0.2 ppm CLEO	1. Increase of 106.4 % and 296.8 % in AOX 2. Decrease of 0.3 and 0.4 log cfu g^{-1} total viable bacteria and 0.1 and 0.2 log cfu g^{-1} yeast and mould count	(Badola et al., 2018)
Herbs *Angelica sinensis* (AS) and *Codonopsis pilosula* (CP)	Chicken seasoning	Shelf-life enhancement	-	Increase of 54 days for AS and 39 days for CP at 25 °C storage	(Tian et al., 2019)
Origanum vulgare	Cucumber	Product quality and shelf life enhancement	10:1 ratio of herb infusion: cucumber in osmotic treatment for 2.5 h at 70 °C	7 days increase in shelf life over untreated cucumber at 37 °C storage	(Giannakourou et al., 2019)
Ethanolic extract of *Syzygium aromaticum*, *Cinnamomum cassia,* and *Origanum vulgare*	Raw chicken meat	Improvement of antioxidant, antimicrobial and shelf life qualities	A mixture of 0.3 % v/w of all three extracts	15 days increase in shelf life over untreated meat at 4 °C storage	(Radha krishnan et al., 2014)
Vitamin D	Whole fat milk	Improvement of functional quality and stability	Oil in water emulsion with overall loading of 0.5 µg mL^{-1} of nanoencapsulation Vitamin D	Stability of fortified milk improved to 10 days at 25 °C storage	(Golfomitsou et al., 2018)
Curcumin from turmeric	Extruded food product	Color stability comparison of the product as against synthetic pigment tartrazine	52.6 ppm curcumin and 76.6 ppm tartrazine	Curcumin could replace tartrazine for a 6 week storage at 27 °C	(Sowbhagya et al., 2005)
Anthocyanin extract from *Kokum* (*Garcinia indica Choisy*)	Watermelon rinds	Improvement of shelf stability	Infusion with 168.6 mg L^{-1} anthocyanin concentration	Product stable up to 90 days	(Bellary et al., 2016)

the quality of ginger endemic to northeast India (Kiran et al., 2013) and the focus of these studies has only been limited to ginger oil and oleoresin characteristics. Hence, the gap can be addressed by presenting a comprehensive database of physico-chemical properties of ginger varieties from northeast India. The database of industrially relevant parameters will serve as a guide for setting up of commercial applications and customized product development research in food/pharmaceutical sector and also in the field of genetic engineering and development of newer ginger varieties with a better combination of quality attributes.

1.8.2 Extraction technology and process parameters

The literature has been found to focus primarily on producing a mixture of ginger extracts containing volatile oil and oleoresin. Only a few reports have focused either on the isolation of volatile oil which comprised of smaller and lighter molecules, usually obtained at lower $SCCO_2$ pressure ranges (below 150 bar), or on oleoresin, which is a thick viscous extract. Fractional separation involving selection of appropriate $SCCO_2$ pressure and temperature, in multiple separators connected in series, has been reported to selectively isolate compounds of interest in a single stage process. However, to the best of the knowledge so far, no reports are available involving $SCCO_2$ either solely for the concentration of gingerols or simultaneous recovery of gingerols enriched oleoresin and volatile oil from the ginger.

Therefore, it is desired to address this gap by developing a single step process for $SCCO_2$ extraction coupled with fractionation to concentrate gingerols in oleoresin and simultaneously recover pure volatile oil from the dry ginger.

1.8.3 Processing of ginger oleoresin for food applications

Despite the available state of the art, the use of ginger oleoresin for non-lipid based food applications largely remains unexplored. Very few reports are available which have attempted to enhance the water dispersibility of ginger actives incorporating a very high surfactant to oil ratio (SOR) such as 16:1 to 66:1. Formulations with such a high SORs may not be fit for food applications owing to regulatory requirements of recommended daily allowance (RDA) limits (Additives and Food, 2015). Moreover, none of these reports have focused on application of ginger oleoresin specifically for

TH-2388_133107006

food product development. It is hence desired to develop a carrier system for gingerols enriched oleoresin with low SOR, tailored for non-lipid based food applications.

1.8.4 Ginger based food product development

It is hypothesized that incorporation of ginger oleoresin in candied mango will synergistically enhance the unique sweet-sour flavor palette of mango fruit based product along with improving its nutraceutical properties. Till date, no systematic reports are available which involve gingerols infusion for development of a candied mango product, its process optimization and scale-up studies. Such studies ultimately help in the commercialization of newer candying techniques and very limited literature is available in this area.

1.8.5 Storage studies

Infusion of gingerols into candied mango and storage in a packaging material having high barrier properties, for the sole objective of enhancing its shelf-life remains an unexplored area. Moreover, accurate prediction of shelf-life employing multivariate techniques for candied fruit based products has also not been studied extensively.

1.9 Specific objectives of thesis

Based on the state of the art and research gaps discussed in above sections, following major objectives have been decided:

- Identification of best ginger varieties from northeast India from the perspective of antioxidant and nutritional potential
- Parametric optimality of supercritical fluid extraction-cum-fractionation technology for isolation of gingerols enriched oleoresin from selected ginger variety
- Design of carrier system for gingerols enriched oleoresin tailored for food applications
- Gingerols infusion and development of mango fruit based ready to eat model food system
- Storage studies for the developed ginger-based products

1.10 Organization of thesis

The contents of thesis are organized in eight chapters as follows:

Chapter 1	Introduction
Chapter 2	Materials and methods
Chapter 3	Identification of best ginger varieties from northeast India from the perspective of antioxidant and nutritional potential
Chapter 4	Parametric optimality of supercritical fluid extraction-cum-fractionation technology for isolation of gingerols enriched oleoresin from the selected ginger variety
Chapter 5	Design of a carrier system for gingerols enriched oleoresin tailored for food applications
Chapter 6	Gingerols infusion and development of mango fruit based ready to eat nutraceutical food product
Chapter 7	Storage studies for the developed gingerols infused food product
Chapter 8	Conclusions and scope for future work

Chapter 1: Introduction

This chapter briefly gives fundamental information about ginger, its chemistry and medicinal properties. It reviews various published literature in the area of quality and characterization of different ginger varieties found around the world. Following this, various techniques and process parameters involved in the processing and extraction of antioxidants from ginger are presented. State of art on processing of ginger extract, its application in food product development as well as shelf life studies are also discussed. The review of literature follows to the research gaps and finally specific objectives and organization of thesis are presented.

Chapter 2: Materials and methods

This chapter details all the analytical procedures and protocols employed in the characterization, experimentation and theoretical work done in the thesis. The chapter starts with details of collected ginger from different parts of northeast India along with its geographical markers. Following this, methods utilized for characterization of various ginger

TH-2388_13207006

varieties in terms of their nutritional and antioxidant potential collected are presented. It also gives details pertaining to the methodologies and apparatuses employed in laboratory scale extraction, carrier design product development and shelf life studies undertaken in the thesis work. Particulars of commercial scale facilities, procedures and scale-up trial details have also been discussed. Specifications of all raw materials, chemicals and reagents used throughout the thesis work have also been documented.

Chapter 3: Identification of best ginger varieties from northeast India from the perspective of antioxidant and nutritional potential

This chapter attempts to present the details of collected ginger varieties from diverse regions of northeast India and conduct a detailed and systematically study of its physico-chemical properties. The chapter starts with details of geographical markers of collected ginger varieties along with physical parameters and morphological properties of different kinds of rhizomes collected for each variety. The discussion follows to physico-chemical characteristics comprising of detailed micronutrient compositions of 11 essential minerals and macronutrient compositions of 8 parameters for each variety. Antioxidant parameters in terms of extractives in different solvents, their antioxidant capacities, total phenolic and flavonoid contents have been discussed. Detailed chemical characteristics of extracted oleoresin in terms of 6-gingerol contents and individual volatile components of extracted essential oil has been presented along with 4 industrially important physical properties for each oleoresin and essential oil samples. Finally, a screening methodology for selecting best ginger variety among a pool of different varieties for industrially relevant parameters is presented employing multivariate data analysis tools and correlation analysis.

Chapter 4: Parametric optimality of supercritical fluid extraction-cum-fractionation technology for isolation of gingerols enriched oleoresin from selected ginger variety

This chapter focuses on developing a single step green process for supercritical CO_2 ($SCCO_2$) extraction coupled with fractionation of dry ginger for simultaneous production of gingerols rich oleoresin and volatile oil. The optimization and scale-up

validation of the process developed have been presented in three stages. Firstly, $SCCO_2$ extraction conditions optimized for the highest yield of mixture extract containing a maximum amount of volatile oil components and major actives, under laboratory scale conditions are presented. Thereafter the results of scale-up validation of these conditions on a commercial scale unit are presented. Finally, results of online $SCCO_2$ fractionation experiments conducted at same optimum extraction conditions for simultaneously separating high quality oleoresin and volatile oil fractions are presented. The yield and quality of oleoresin and volatile oil extracted from conventional methods have also been compared.

Chapter 5: Design of a carrier system for gingerols enriched oleoresin tailored for food applications

This chapter attempts to develop a stable formulation of ginger oleoresin with enhanced water dispersibility, with a low surfactant to oleoresin ratio, targetted specifically for water based food fortification applications. A range of food-grade excipients with bioenhancer properties were employed for developing the formulation. Results of various experimental methodologies are presented for arriving at the optimum ratio of all excipients for highest oleoresin solubility as well as loading, resulting in dispersion having minimum particle size. There after results of the *in-vitro* release study as well as stability and long term storage study for the developed formulation are presented.

Chapter 6: Gingerols infusion and development of mango fruit based ready to eat nutraceutical food product

This chapter presents the results of application of the developed formulation for development of a functional food product. The chapter starts with results of the preliminary experiments aimed at testing the formulation for incorporation of gingerols in fresh mango based candied product developed at laboratory scale. Thereafter, the scale-up validation along with results of undertaking various process improvement measures under commercial scale settings are discussed. Finally, critical quality and

TH-2388_133107006

mass transfer parameters of laboratory scale and scale-up experiments are compared with a similar commercial product manufactured under similar commercial scale settings.

Chapter 7: Storage studies for the developed gingerols infused food product

This chapter presents the shelf life behaviour of the developed candied mango product under different conditions of temperature and packaging materials under accelerated storage conditions. The chapter also attempts to present a multivariate data analysis based methodology for accurate shelf-life prediction of industrial food products. Firstly, the kinetic behaviour of a range of parameters are discussed and shelf life predictions are made based on conventional kinetics approach of all the individual quality parameters. There after the effect of collated interactive behaviours of various parameters on storage behaviour is presented and shelf-life predictions are made based on multivariate data analysis. Finally, the predictions were compared with the actual shelf-life of a similar commercial product, when stored under normal storage and packaging conditions.

Chapter 8: Conclusions and scope for future work

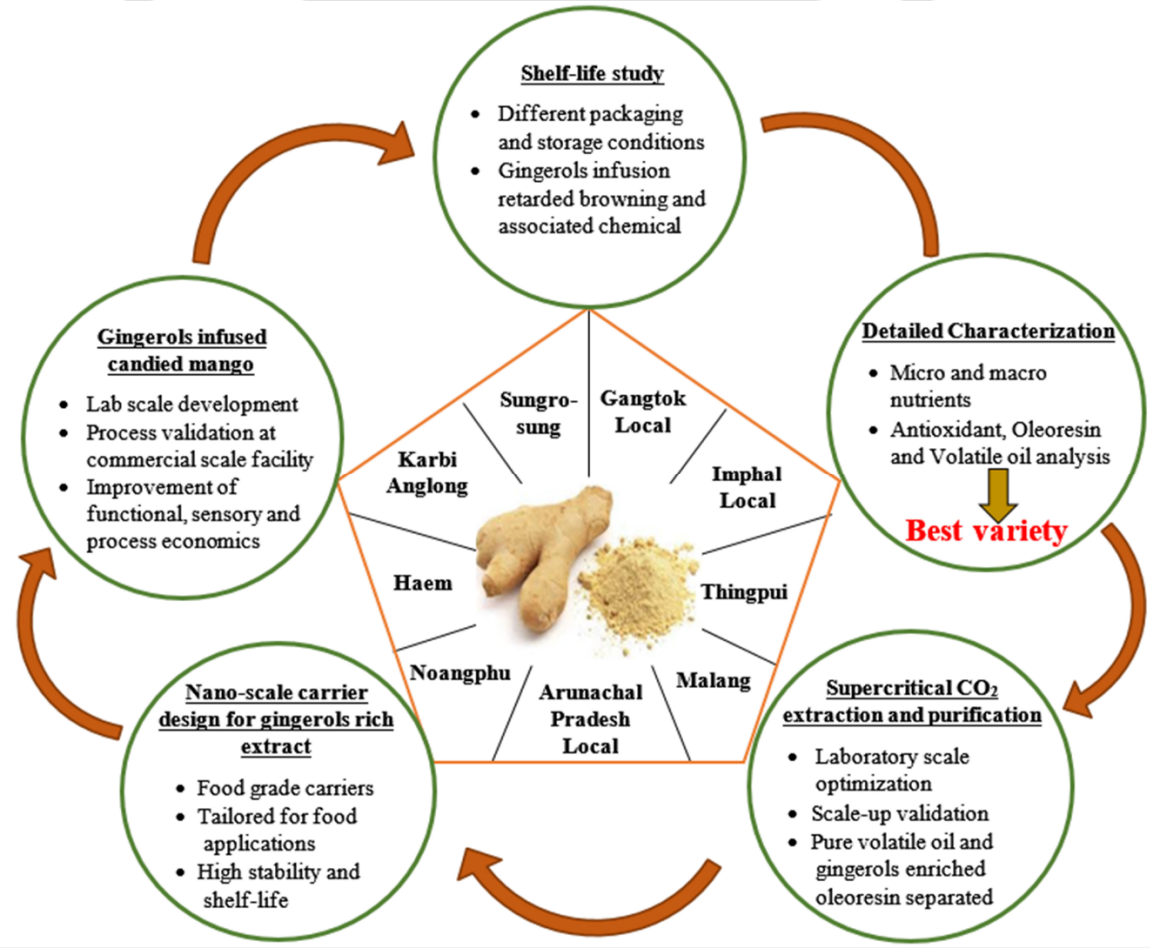
This chapter presents conclusion drawn from the research work. It also provides possible direction and recommendation for future work based on the output of thesis work.

TH-2386_136107006

Chapter 2

Materials and methods

This chapter details all the analytical procedures and protocols employed in the characterization of the samples. It also gives details pertaining to the methodologies and apparatus employed in laboratory scale extraction, carrier design product development and shelf life. Particulars of commercial scale facilities, procedures and scale-up trial details have also been discussed. Specifications of all the raw materials, chemicals and reagents used throughout the thesis work have also been documented.



TH-2386_136107006

TH-2386_136107006

2 Materials and methods

2.1 Collected ginger varieties

Nine different cultivated varieties of ginger (*Zingiber officinale*) were collected from all states of the northeast region of India. Their local names were *Gangtok local* from Sikkim, *Imphal local* from Manipur, *Arunachal Pradesh local* from Arunachal Pradesh, *Thingpui* from Mizoram, *Sungro-sung* from Nagaland, *Karbi Anglong* from Assam, *Haem* and *Malang* from Meghalaya and *Nongphu* from Tripura. All of these ginger varieties were harvested in the year 2014. **Fig. 2.1** and **Table 2.1** give the origin details of the collected samples along with their specific location coordinates. **Fig. 2.2** shows the collected fresh ginger samples.

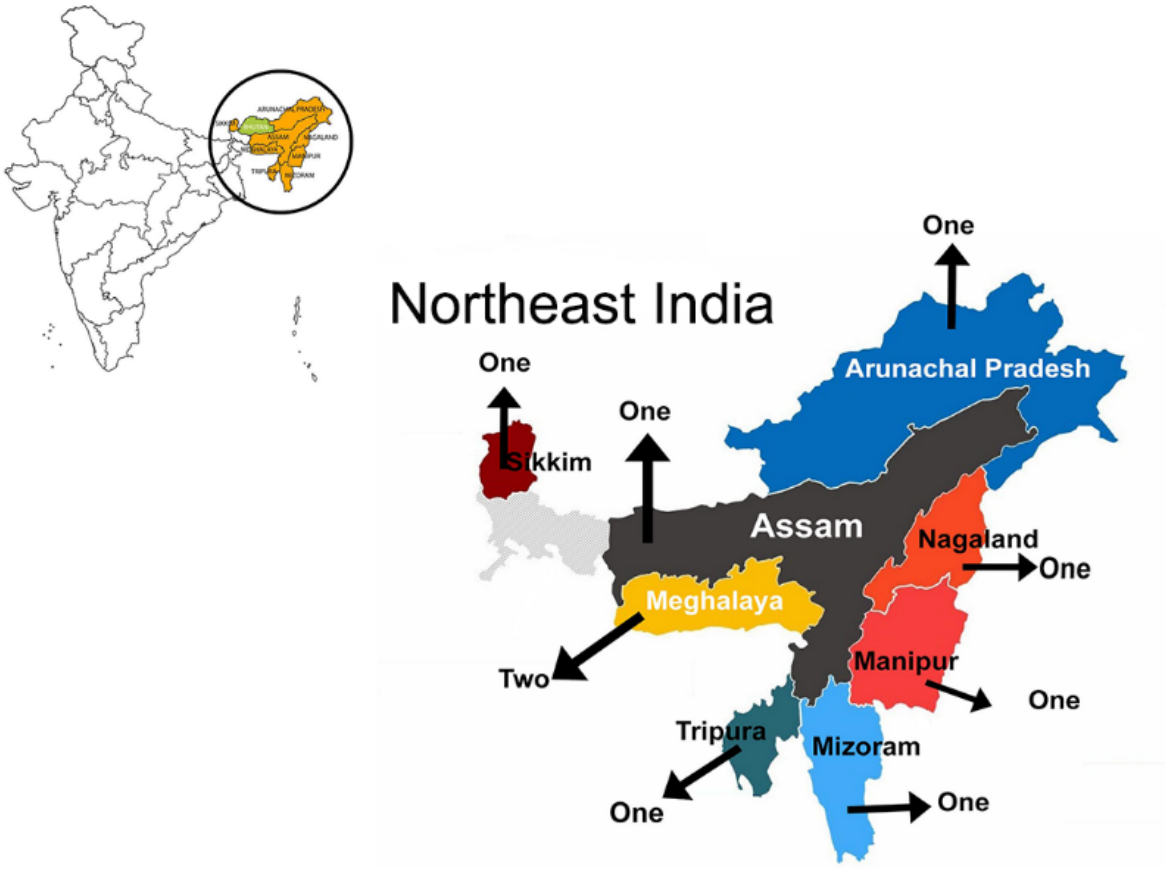


Fig. 2.1: Map showing locations along with a number of collected ginger varieties

Table 2.1: Collected ginger varieties under study and their origin

Sample Code	Variety Name	Origin	Location co-ordinates
1	*Gangtok Local*	Sikkim	27.3389426° N, 88.6064965° E
2	*Imphal Local*	Manipur	24.806311° N, 93.9481849° E
3	*Arunachal Pradesh Local*	Arunachal Pradesh	27.0869089° N, 93.6089019° E
4	*Thingpui*	Mizoram	23.164561° N, 92.9375559° E
5	*Sungro-sung*	Nagaland	26.094397° N, 94.259963° E
6	*Karbi Anglong*	Assam	26.1101164° N, 93.0325765° E
7	*Haem*	Meghalaya	25.747399° N, 91.888947° E
8	*Malang*	Meghalaya	25.6768104° N, 91.9270196° E
9	*Noangphu*	Tripura	23.831465° N, 91.2867697° E

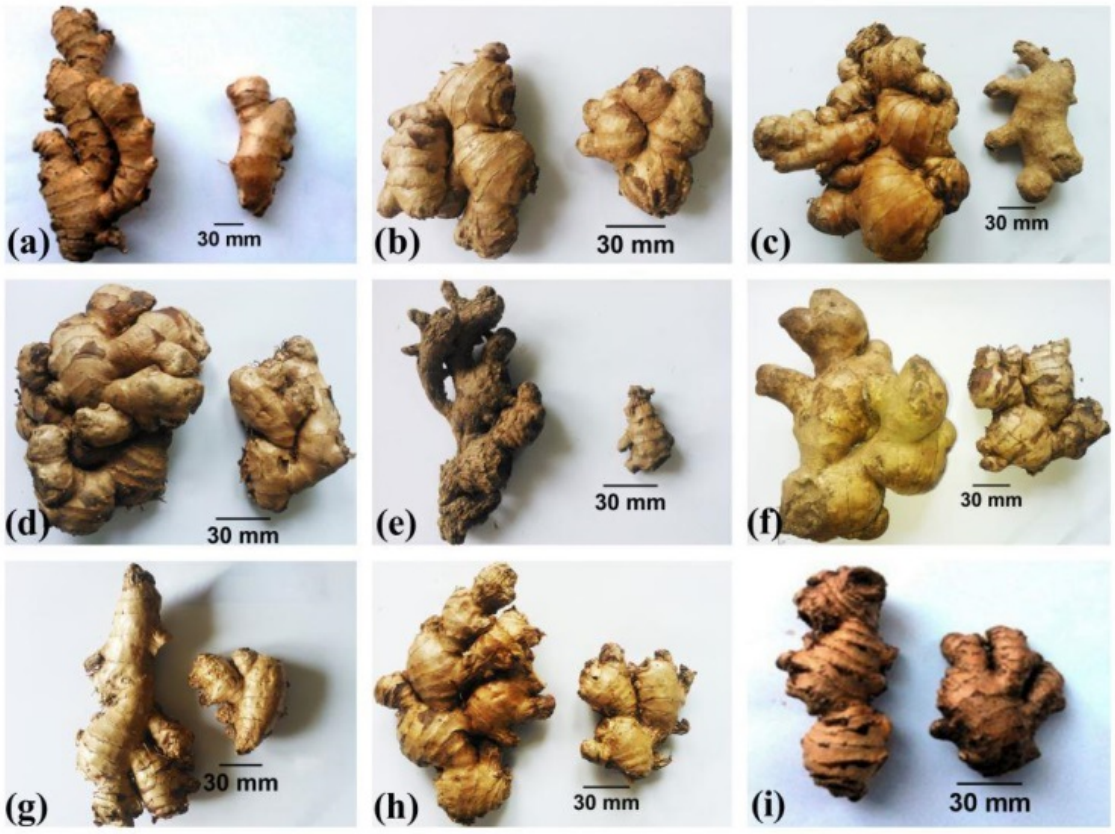


Fig. 2.2: Collected varieties of ginger, a) *Gangtok local,* b) *Imphal Local,* c) *Arunachal Pradesh local,* d) *Thingpui,* e) *Sungro-sung,* f) *Karbi Anglong,* g) *Haem,* h) *Malang and* i) *Noangphu*

TH-2388_13207006

2.2 Chemicals and reagents

Various chemicals and reagents used for analytical experiments conducted throughout the thesis are given in **Table 2.2** along with the details of their purity, grade and make. These chemicals/reagents were used as received throughout the thesis work.

Table 2.2 (a): Details of chemicals/reagents under study

Reagents/chemicals	Purity (%)	Grade	Make
6-gingerol	99	HPLC	Sigma-Aldrich (USA)
β-carotene	≥98	AR	Axiva Life Sciences (India)
Vitamin C	≥98	AR	Axiva Life Sciences (India)
Gallic acid	≥98	AR	Merck (India)
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	≥98	AR	Merck (India)
H_2SO_4	≥95	AR	Sigma-Aldrich (USA)
NaOH	≥98	AR	Merck (India)
Ethyl Alcohol	≥95	AR	Changshu Yangyuan (China)
Methyl red indicator	≥98	AR	Axiva Life Sciences (India)
Potassium Sulphate	≥98	AR	Axiva Life Sciences (India)
Phenolphthalein indicator	-	AR	Axiva Life Sciences (India)
$HClO_4$	≥98	AR	Merck (India)
Folin-Ciocalteu (FC) reagent	-	AR	Merck (India)
$AlCl_3$	≥98	AR	Merck (India)
Quercetin	99	HPLC	Sigma-Aldrich (USA)

Table 2.2 (b): Details of chemicals/reagents under study

Reagents/chemicals	Purity (%)	Grade	Make
2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [ABTS]	≥98	AR	Sigma-Aldrich (USA)
Orthophosphoric acid	≥98	AR	Merck (India)
Metaphosphoric acid	≥98	AR	Merck (India)
Methanol	99	HPLC	Merck (India)
Acetonitrile	99	HPLC	Merck (India)
Sucrose	≥95	Commercial	Mawana (India)
2, 6-dichlorophenolindophenol	≥98	AR	Merck (India)
Kjeldahl tablets	≥95	AR	Merck (India)
Hexane	≥98	AR	Merck (India)
Ethyl Acetate	≥98	AR	Merck (India)
Petroleum ether	≥98	AR	Merck (India)
α-amylase	≥98	AR	Sigma-Aldrich (USA)
pH buffer solutions	-	AR	Sigma-Aldrich (USA)
$CaCl_2$	≥98	AR	Sigma-Aldrich (USA)
Glucose	≥98	AR	Sigma-Aldrich (USA)
HCl	≥98	AR	Merck (India)
Na_2CO_3	≥98	AR	Merck (India)
Anthrone reagent	≥98	AR	Merck (India)
Lead acetate	≥98	AR	Merck (India)
Potassium oxalate	≥98	AR	Merck (India)
Methylene Blue indicator	-	AR	Axiva Life Sciences (India)

TH-2388_13307006

2.3 Characterization of collected varieties

2.3.1 Physical parameters

Fresh ginger rhizomes of all varieties were washed, blotted with tissue paper to remove adhering moisture and used for finding physical parameters.

Moisture Content

The rhizomes were cut into thin slices using a knife and dried in a hot air oven at 50 ^{o}C for 24 h until the weight change was constant for determining dry matter. Average moisture content was estimated using the following equation.

$$Moisture\ content = \frac{Weight\ of\ dried\ matter}{Total\ weight\ of\ fresh\ rhizome} \times 100 \quad (2.1)$$

Edible index, average size and weight

Edible index, average size and weight were calculated using method described by (Hazarika and Kakoti, 2013). Average weight was calculated using analytical weight balance by selecting 10 rhizomes randomly. The spatial dimensions (length, breadth and thickness) of ginger rhizome were determined using Vernier calipers scale having least count of 0.1 mm. Average size was the geometric mean of the above three dimensions and calculated using the following expression.

$$Size = \sqrt[3]{length \times breadth \times thickness} \quad (2.2)$$

The non-edible part was removed from the rhizomes using a knife and the edible index was calculated using following expression:

$$Edible\ Index = \frac{Weight\ of\ edible\ portion\ of\ rhizome}{Total\ weight\ of\ rhizome} \times 100 \quad (2.3)$$

Water retention capacity (WRC) and swelling capacity (SC)

Water retention and swelling capacity of all the varieties were estimated on dry weight basis following the method described by Das et al., (2016). For swelling capacity,

100 mg dried ginger powder was soaked in 40 mL DI water. The mixture was stirred occasionally and kept for 18 h to attain equilibrium. The change in volume was recorded and expressed as mL $H_2O.g^{-1}$ dry powder.

Similarly, 100 mg dried ginger powder was soaked in 20 mL water for 24 h. The soaked ginger powder was separated and centrifuged at 4000×*g* for 30 min. The supernatant was filtered and residual solids were weighted, dried at 100 ºC for 24 h and weighted again. The WRC was expressed as water retained per gram of dry ginger powder.

2.4 Chemical parameters

The fresh ginger rhizomes of all the varieties were dried to a moisture content of 12 ± 2%, powdered and stored in airtight bags in the refrigerated condition for all further analysis.

2.4.1 Macronutrients

Crude fiber

AOAC method No. 962.09 was used to estimate crude fiber contents in different ginger varieties. 10 gm ginger powder was accurately weighted and transfer to a thimble and extracted in a Soxhlet extractor with petroleum ether for defatting. Extracted sample was air dried at 25 °C and transferred to dry 1 L conical flask. Sulphuric acid solution (strength 1.25 %) was transferred to the flask containing the defatted material and connected with a water cooled reflux condenser. The heater connected to the flask was switched on and the contents of the flask were made to boil for 30 min. After cooling and removing the flask, the contents were filtered through crucible and washed with boiling water until the washings were no longer acid to litmus. After this step, the residue was washed with 200 mL of boiling sodium hydroxide solution (strength 1.25 %). Thereafter, the contents were again boiled in deionized water (DI water) with a reflux condenser for 30 min. After boiling and cooling, residue was thoroughly washed first with boiling water and then with ethyl alcohol and transferred to a Gooch crucible prepared with a thin compact layer of ignited asbestos. Gooch crucible and contents were dried at 105 ± 2 °C in a hot air oven until constant weight was achieved and weighted. After this step, the contents of the Gooch crucible were incinerated in a muffle furnace at 130 °C for 2 h until all carbonaceous matter was burnt. After this, the Gooch crucible containing ash was cooled in a desiccator and weighted. Crude fibre was thus calculated by following

TH-2388_13307006

formula.

$$Crude\ fibre, \%\ by\ wt. = \frac{W_1 - W_2}{W} \times 100 \quad (2.4)$$

Where,

W_1= wt. in g of Gooch crucible and contents before ashing

W_2 = wt. in g of Gooch crucible containing asbestos and ash

W = wt. in g of the dried material taken for the test

Crude protein

Crude protein contents were estimated following the AOAC methods number 960.52. 1 g of the sample was weighted and transferred to 800 mL Kjeldahl flask. To this Kjeldahl tablets were added (Composition: 48.8 % Sodium Sulphate, 48.9 % Potassium Sulphate, and 0.3 % Copper Sulphate) along with glass beads. After this, the flask was placed for digestion in a digestion chamber at an inclined position. The flask was gently heated at low flame until the initial frothing ceased and the mixture boiled steadily at a moderate rate for around 1 h until the colour of the digest turn to pale blue. After this, the digest was cooled and 200 mL of water was added slowly. Thereafter, sufficient amount of sodium hydroxide solution (450 $g.L^{-1}$) was carefully added to make the contents strongly alkaline (about 110 mL) before mixing the acid and alkaline layer. After this, the flask was connected to a distillation apparatus with a condenser. To the condenser, a delivery tube was fitted which dipped just below the surface of the pipetted volume of standard acid contained in a conical flask receiver. The contents of the digestion flask were mixed and boiled until 150 mL distilled into the receiver. Around 5 drops of methyl red indicator were added and titrated with standardized 0.1 N sodium hydroxide solution. Blank titrations were carried out simultaneously. Protein calculations were estimated as per following equations:

Considering 1 mL of 0.1N H_2SO_4 = 0.0014 gm Nitrogen,

$$Protein\ content = Nitrogen\ content \times 6.25 \quad (2.5)$$

$$\%\ Protein\ content\ (dry\ wt.\ basis) = \frac{Protein\ content}{100 - Moisture\ content} \times 100 \quad (2.6)$$

Total ash

Total ash content was estimated according to AOAC method No. 923.09. The dried sample left after determination of moisture was put in a muffle furnace at 550 ± 60 °C till a grey ash was obtained. It was cooled in a desiccator and weighed. It was heated again in the muffle furnace for 30 min, cooled and weighed. This was repeated until the two successive weights were constant. The lowest weight was recorded and this was the total ash on dry weight basis. Calculations involved are as follows.

$$\%\ Total\ Ash\ content\ (dry\ wt.\ basis) = \frac{W_2 - W}{W_1 - W} \times 100 \qquad (2.7)$$

Where, W_2 = wt. of dish with the ash (in g)

W = wt. of empty dish (in g)

W_1 = wt. of dish with dried sample (in g)

Fat

Fat content were estimated employing AOAC No. 960.39 method. 10 g of dried ginger powder samples were filled in a thimble and extracted in a Soxhlet apparatus using petroleum ether for 8 h. After complete extraction the sample was cooled to room temperature (28 ± 2 °C) and the excess solvent was recovered using rotary evaporator. The final solid obtained after evaporation was weighed for estimation of fat content.

Starch and total carbohydrates

Estimation of starch was carried out by Anthrone reagent method (Thayumanavan and Sadasivam, 1984). Small amount of sample (0.5 g) was mixed properly in hot 80 % ethanol to remove sugars. This mixture was centrifuged at 4000 × *g* for 10 min and residue was separated. The process was repeated till the supernatant did not give color with Anthrone reagent. The separated residue was dried using a water bath. To this residue, 5.0 mL of water and 6.5 mL of 52 % perchloric acid were added and kept at 0 °C for 20 min. This mixture was centrifuge at 4000 × *g* for 10 min and supernatant was separated. The process was repeated 4-5 times and supernatant was collected and was finally made up to 100 mL. From this, 0.1-0.2 mL of the supernatant was taken and its volume made up to 1 mL with distilled water. Along with this, standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of glucose standard (100 mg dissolved

TH-2388_133107006

in 100 mL DI water). Volume of each test tube was made up to 1mL. Supernatant was filtered and aliquots was taken for analysis and added with 4 mL of Anthrone reagent in each test tube. It was heated for 10 min in a boiling water bath and cooled rapidly and the absorbance was read at 630 nm. The amount of glucose content in the sample was calculated using standard graph and was multiplied with a factor of 0.9 to get the starch content in the sample (detailed discussion presented in Appendix 1).

Carbohydrate was estimated in a similar way as per Anthrone method (Thayumanavan and Sadasivam, 1984). 100 mg of the sample was hydrolyzed by keeping it in boiling water bath for 3 h with 5 mL of 2.5 N-HCl and then cooled to room temperature (28 ± 2 °C). This was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 mL and centrifuged at 4000 × *g* for 10 min. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the glucose standard (100 mg dissolved in 100 mL DI water). Volume of each test tube was made up to 1 mL including the sample tubes by adding distilled water. Supernatant was filtered and aliquots was taken for analysis and added with 4 mL of Anthrone reagent in each test tube. It was heated for 10 min in a boiling water bath and cooled rapidly and the absorbance was recorded at 630 nm. The amount of carbohydrate present in the sample was calculated as per the following equation.

$$Total\ carbohydrates\ (in\ 100mg) = \frac{Amount\ of\ glucose\ in\ sample}{Weight\ of\ sample} \times 100 \qquad (2.8)$$

Total and reducing sugars

Total and reducing sugars were estimated as per AOAC method Nos. 923.09. Weighed sample of 10 g was dissolved in water and volume made to 250 mL in a conical flask. To this was added 2 mL of lead acetate solution, shaken well, and kept for 10 min. Necessary amount of potassium oxalate was added to remove the excess of lead and filtered through Whatman filter paper No. 1. The filtrate was used for the estimation of reducing sugars. In a conical flask, 5 mL each of Fehling's solution A (prepared by mixing 7 g of $CuSO_4$ in 100 mL distilled water with 2 drops of dilute Sulphuric acid) and B (prepared by mixing 35 g of potassium tartrate and 12 g of NaOH in 100 mL of distilled water) were taken. The sugar extract was titrated against boiling Fehling's solution by using methylene blue as an indicator. The end point was indicated by the appearance of

brick red precipitates.

$$\%\ Reducing\ sugars\ =\ \frac{Amount\ of\ invert\ sugar\ \times\ Dilution}{Titre\ \times\ Weight\ of\ sample}\ \times 100 \qquad (2.9)$$

For standard invert sugar solution, 9.5 mg sugar was dissolved in 100 mL water and 5 mL concentrated HCl. This was allowed to stand for 3 days at room temperature (28 ± 2 °C) for sugar inversion and made up to 1 L by adding water. Amount of invert sugar in the formula was determined by titrating equal amounts of Fehling's A and B with the prepared invert sugar solution. The end point was reached at complete decolourization of the indicator.

$$Amount\ of\ invert\ sugar\ =\ \frac{Titre\ \times\ 2.5}{1000} \qquad (2.10)$$

For total sugars, 50 mL of the extract was taken in a 100 mL volumetric flask to which 1.0 mL concentrated HCl was added and kept for hydrolysation overnight at room temperature (28 ± 2 °C). Next day, the solution was neutralized with saturated NaOH solution followed by a drop of phenolphthalein, finally the volume was made up to the mark with distilled water. This solution was then titrated against Fehling's A and B as was done previously in case of reducing sugars. The titre volume was used to calculate the per cent total sugar using the formulae.

$$\%\ Total\ sugars\ =\ \frac{Amount\ of\ invert\ sugar\ (mg)\ \times\ Dilution}{Titre\ \times\ Weight\ of\ sample\ (g)}\ \times 100 \qquad (2.11)$$

2.4.2 Micronutrients

Flame photometer (Model: Systronic 128, Make: Systronic India, Delhi, India) was used for estimation of Na, K, P. The concentrations of Mg, Ca, Fe, Mn, Zn, Cr, Ni, and Cu was estimated by atomic absorption spectrophotometer (Model: Spectra AA 220 FS; Make: M/s Varian, Netherland). 0.2 g of dry sample was digested with 10 mL of a mixture of H_2SO_4 and $HClO_4$ (5:1 ratio) in block digestion system (Pelican Equipments, Chennai, India) for 2 h at 300 ^oC.

TH-2388_13307006

2.4.3 Antioxidant parameters

Solvent extractives were estimated by taking 100 g of dried and powdered ginger with a range of solvents in increasing order of their polarity. The solvent to feed ratio was kept at 8:1 for 10 h in a Soxhlet apparatus (Das et al., 2017). The extracts were separated from the extract-solvent mixtures by using laboratory grade rotary evaporator (R-100, Buchi Labortechnik, Switzerland) and stored in amber bottles in the refrigerated condition for further use. Amount of extract recovered from each solvent was designated as the percentage yield in that solvent. The ethanolic extracts were used for estimation of antioxidant parameters.

Total phenolic content (TPC) and total flavonoid contents (TFC)

Total phenolic content was estimated using Folin-Ciocalteu (FC) assay as per the method described by Das et al., (2017). A known amount of extract (10 mg mL^{-1}) was mixed with 1.0 mL of FC reagent and 0.8 mL of 2 % Na_2CO_3 was added and the volume was made up to 10 mL using water- methanol (4:6) as diluting fluid. Absorbance was read at 740 nm after 30 min using spectrophotometer. Gallic acid (0 - 800 mg L^{-1}) was used to produce standard calibration curve (Appendix 2). The total phenolic content was expressed in mg of Gallic acid equivalents (GAE) per 100 g of sample.

The total flavonoid content was determined in a similar way as per the method described by Arvouet-Grand et al., (1994). A 5.0 ml of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (10 mg mL^{-1}). Absorption readings at 415 nm using UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of extract solution with 5.0 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin (Appendix 3) and expressed as g of quercetin equivalents (QE) per 100 g of sample.

Total antioxidant activities

Radical scavenging activity (RSA) in terms of free radicals, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) were carried out following the methods of (Carmona-Jiménez et al., 2014) and (Shahidi and Zhong, 2015) respectively. Positive control (standard) was prepared by mixing 4.0 mL of ascorbic acid (0.05 mg mL^{-1}) and 1.0 ml of DPPH (0.4 mg mL^{-1}) for water extract, and negative control (blank) was prepared by mixing extract base (ethanol) with 1.0 mL of DPPH. Four different concentrations of extract were mixed with 4.0 mL of DPPH, the

volume made up to known volume, mixed well and left to stand at room temperature in a dark place for 30 min. Absorbance (OD) was read using a spectrophotometer at 520 nm for DPPH and 750 nm for ABTS. The ability of extract to scavenge both radicals in terms of % inhibition was calculated using the following equation:

$$\% \, Inhibition = \frac{Control \ OD - Sample \ OD}{Control \ OD} \times 100 \qquad (2.12)$$

Gingerols estimation

Sum total of 6, 8, 10 gingerols and 6-shogaol were expressed as major actives and their quantitative estimation in the extract was done using HPLC system (model: Prominence, make: M/s Shimadzu, Singapore) equipped with Diode Array Detector (DAD) having a C18 column (250µm × 4.6µm, 5µm). The analysis was conducted using a gradient system mobile phase having a mixture of 0.1% orthophosphoric acid: acetonitrile in the ratio of 35:65. The flow rate of mobile phase was kept at 1.0 mL min^{-1} and detection was done on UV absorption at 280 nm. The extract was suitably diluted and 20 µl volume was injected at ambient temperature. Major actives namely: 6, 8, 10 gingerols and 6 shogaol were identified and quantified based on the peaks and retention time of the mixed standard chromatogram (Fig. 2.3).

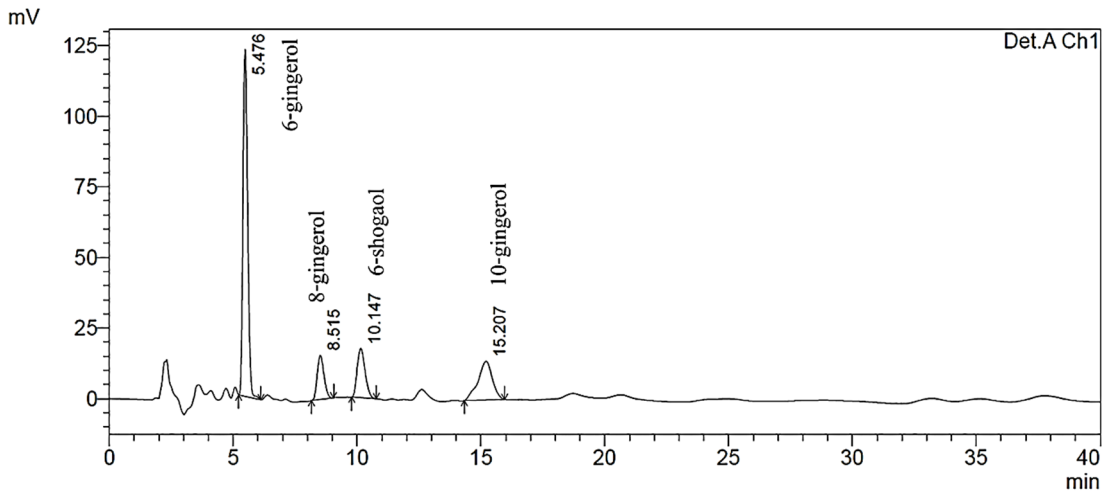


Fig. 2.3: HPLC Chromatogram of gingerols standard.

Essential oil estimation

Essential oil was extracted from 100 g dry ginger powder by steam distillation

TH-2388_13307006

method using a Clevenger type apparatus connected to condenser. The volatile oil collected above the water in the fractionating arm was considered as the yield.

Essential oil characterization

The volatile oil was examined on a Varian 3900 gas chromatograph equipped with a Flame Ionization detector (FID) and a Varian Saturn 2100T mass spectrometer (make: M/s Varian, California, USA) as per the method described by Ravi Kiran et al., (2013). The column used for chromatographic separation had following specifications- model: DB-5 (30 m × 0.25 mm internal diameter, 0.25 µm film thickness), M/s Varian, California, USA. Operating conditions used were: injection volume of the sample 1 µL, oven temperature programming 60 °C to 240 °C at 3 °C min^{-1}, carrier gas helium, flow rate 1 mL min^{-1}, split ratio 1:20, injection temperature 240 °C, detection temperature 280 °C. The MS parameters were: 70 eV of ionization voltage, mass range of 40–450 m/z and scan interval of 0.5 s. The compounds were identified by retention indices and compared with the mass spectral library data, NIST 2.0 and Saturn library along with the published data (Raina et al., 2005; Ravi Kiran et al., 2013).

2.4.4 Physical properties of oleoresin and essential oil

Optical rotation, refractive index and specific gravity

Optical rotation was measured with the help of a polarimeter as per AOAC method No. 920.141. Extracts were suitably diluted with concentration of 1 mg mL^{-1} and filled in the polarimeter tube and optical rotation was measured at 25 ^{o}C and 589 nm wavelength. Similarly, the refractive index was also measured as per AOAC method No. 920.141 at 25 ^{o}C using a refractometer. The specific gravity was measured using standard AOAC method No. 988.06.

2.5 Extraction experiments

2.5.1 Grinding of ginger rhizomes

For laboratory scale experiments, dried ginger rhizomes were ground using a laboratory grade grinder (LB-100, M/s Remi Corporations, India) at 1000 × *g*. The powder was subjected to sieve analysis using laboratory test sieve shaker machine (BST/MSS-4, M/s Bionics Scientific Technologies**,** New Delhi, India), equipped with 0.5 HP motor. Test sieves of 8-inch diameter (I.S.), having mesh sizes 1204-150 µm were

mounted. The powder thus retained in each sieve was designated with its particle size and samples recovered were stored in an airtight polyethylene bags at 4°C until further use. For scale-up experiments, industrial grade pulverizer (DP-20, M/s D. P. Pulverizer Industries, Mumbai, India) was used for powdering the ginger rhizomes.

2.5.2 Preliminary trials and design of experiments

Based on the literature discussed in the introduction section, five process parameters were chosen for the optimization study. These were: ginger particle size, extraction temperature, pressure, CO_2 flow rate, and extraction time. Particle size and temperature were optimized based on preliminary trials which were performed at fixed $SCCO_2$ extraction conditions of pressure 200 bar, flow rate 40 g min^{-1} and extraction time of 180 min. For optimum particle size, extractions were done at a fixed temperature of 40 °C, using ginger powder samples having a different range of particle size (based on the sieve analysis). For optimum process temperature, extractions were carried out at 35, 40, 45 and 50 °C using optimum particle size.

The remaining parameters were optimized based on a full factorial rotatable centre composite design (RCCD). The process parameters (independent variables) taken was $SCCO_2$ pressure (A) in the range of 150-300 bar, flow rate (B) of 20-40 g min^{-1} and extraction time (C) of 60-180 min (where A, B, and C are real factors). The range was selected based on the reported optimum process conditions of both kinds of extracts (non-volatile and volatile fractions) from ginger for maximum extraction of volatile oil components and oleoresin (Balachandran et al., 2006; Bartley and Foley, 1994; Said et al., 2015; Salea et al., 2017; Zancan et al., 2002). The independent variables were coded at five levels (-1.682, -1, 0, +1, +1.68) and were used to generate experimental design which suggested twenty experiments with eight factorial points, six axial points and six center points by design expert software (version 7.0.0). The extract obtained from each experimental point was analyzed for total yield (%), wt % of major actives and wt % of volatile oil (dependent variables), which were chosen as the responses. The experimental data for each of the responses were fitted based on the following equation (Eq. 1), to form a second order polynomial equation.

$$X = \alpha_o + \sum_{i=1}^{n} \alpha_i Y_i + \sum_{i=1}^{n} \alpha_{ii} Y^2{}_i + \sum \sum_{i>j}^{n} \alpha_{ij} Y_j \qquad (2.13)$$

TH-2388_13407006

Where, x represented response, α_o, α_i, α_{ii} and α_{ij} represented the coefficients of constant, linear, quadratic and interaction terms, respectively. Y_i and Y_j represented coded independent variables (pressure [A], flow rate [B], and time [C]). Experiments were performed at the center points for estimation of data fitting accuracy of the selected models. To eliminate the chances of errors in measurements due to bias, all the experiments were executed in random order. The estimation of model coefficients was performed using multiple regression analysis and was validated by the analysis of variance (ANOVA) results. The optimal conditions were obtained by adopting the optimality (maximality) of desirability function tool for maximum yield of mixture extract, having maximum contents of major actives followed by the volatile oil, in the Design Expert software. All the experiments were replicated three times and reported as average values along with their standard deviation.

2.5.3 Laboratory scale supercritical CO_2 *extraction*

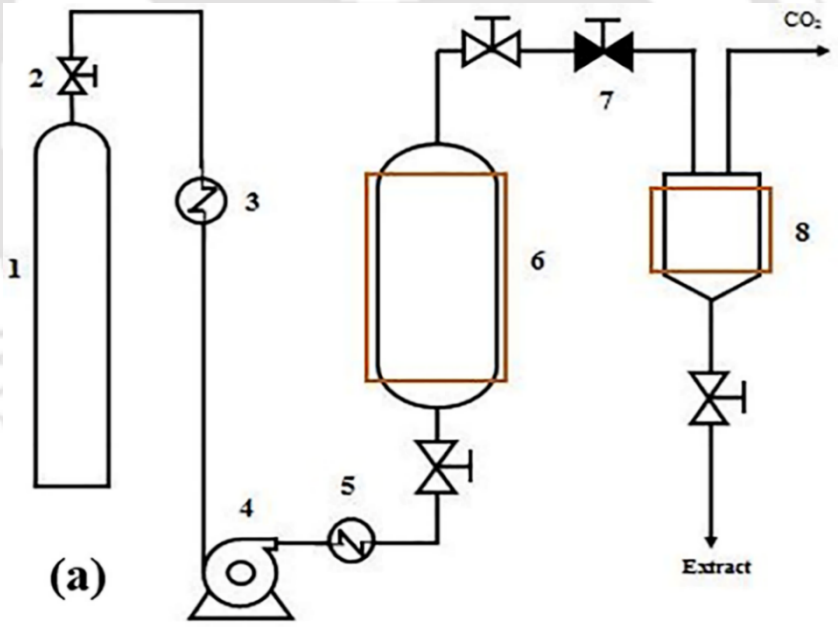


Fig. 2.4(a): Laboratory scale SCFE unit schematic diagram. 1. CO_2 cylinder, 2. Valve, 3. Cooling heat exchanger, 4. CO_2 pump, 5. Heating heat exchanger, 6. Extractor vessel, 7. Automatic back pressure regulator (ABPR), 8. Separator

A laboratory scale supercritical fluid extraction apparatus (SFE 500, Thar Technology, USA) having one extraction vessel (internal diameter, d_i = 60 mm and

height, h = 200 mm) and one separation vessel, each of 500 mL capacity was used (**Fig. 2.4a**). The heat was supplied to the extraction vessel via the electric heating coil, and the temperature was maintained using a thermostat. The pressure was regulated by an automatic back pressure regulator. Process variables such as pressure, flow rate, extraction temperature and time were controlled by program logic control (PLC). One hundred grams of ground ginger rhizomes (density 0.46 g cm^{-3}) was put into a muslin cloth bag and loaded in the extraction vessel. The remaining dead space inside the extraction vessel was filled with glass beads (2 mm diameter, density 2.5 g cm^{-3}), which had a total mass of 600 g.

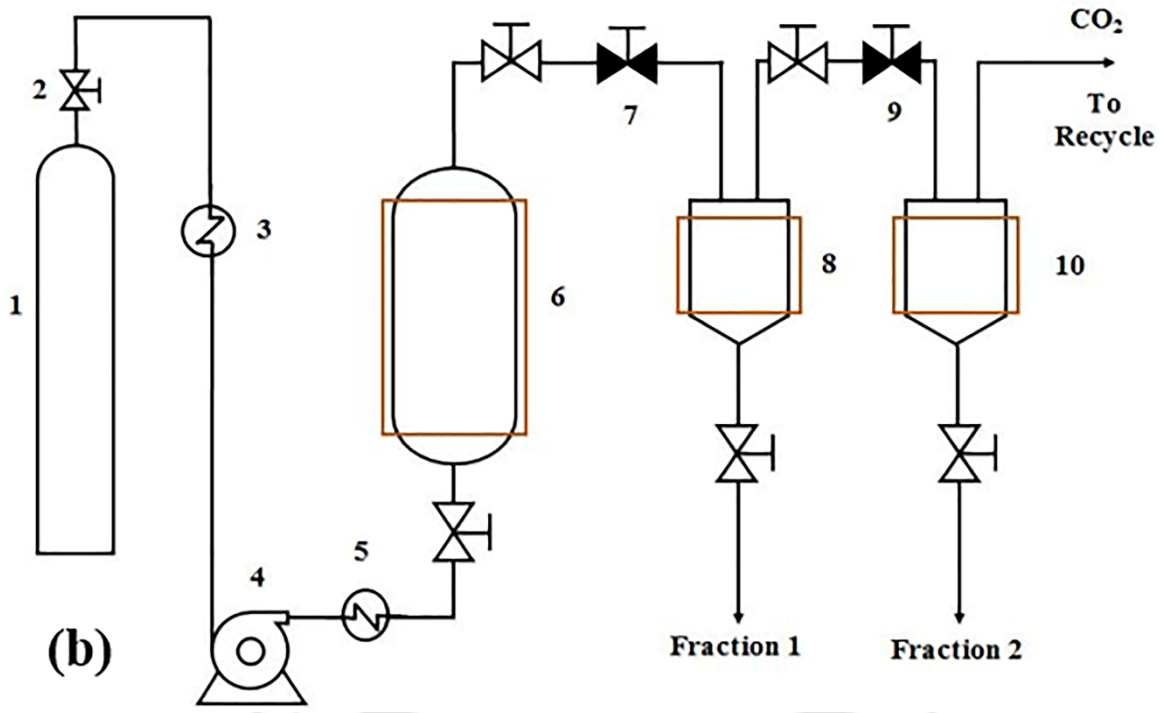


Fig. 2.4(b): Commercial scale SCFE unit schematic diagram. 1. CO_2 cylinder, 2.Valve, 3. Cooling heat exchanger, 4. CO_2 pump, 5. Heating heat exchanger, 6. Extractor vessel, 7 and 9. Automatic back pressure regulator (ABPR), 8. Separator-1 and 10. Separator-2

The $SCCO_2$ was introduced in the extractor where it dissolved the extract from ginger powder. This $SCCO_2$-ginger extract mixture was then introduced to the separator where it was depressurized to atmospheric pressure where CO_2 was released. The extract precipitated was recovered at the bottom of the separator. The weight of extract thus obtained was recorded as percent yield. The extract was collected in airtight amber

TH-2388_134107006

bottles and stored in a refrigerator (4 °C) for further analysis. The volatile oil content in the mixture extract was determined gravimetrically as described by Povh et al. (2001). Briefly, the mixture extract was weighted accurately in a petri dish and dried in a vacuum oven at 25 °C until constant weight was observed. The difference in the weights was used to calculate the percentage of volatile oil content in the extract.

2.5.4 Scale-up experiments

A commercial-scale SCFE facility having three extraction vessels of 25L capacity each (with internal diameter, d_i = 152.4 mm and height, h = 1219.2 mm) and two separator vessels of 5 L capacity, each, connected in series, was used for scale-up experiments (**Fig. 2.4b**). The unit was supplied with pressurized food grade CO_2 through a high-pressure pump. Four kg of dried ginger powder was used for each experiment of extraction and separation. All the process parameters were controlled by PLC.

2.5.5 Supercritical CO_2 extraction coupled with online fractionation

Optimum process conditions resulting in the highest yield of mixture extract (having maximum actives and volatile oil contents) was used for extraction in the commercial scale unit involving two separators. This extract was then fractionally separated by maintaining different sets of higher pressure at separator 1 for recovery of heavier compounds and lower pressure in separator 2 for recovery of lighter volatile components. The saturated $SCCO_2$-extract mixture from the exit point of the extraction vessel first reached separator 1 which was maintained at a pressure lower than the extraction vessel. The change in operating pressure reduced the solubility of some ingredients in the mixture of $SCCO_2$ and extract, causing them to precipitate in separator 1 (Reverchon and De Marco, 2006). The mixture of $SCCO_2$ and remaining part of extract then reached separator 2 which was maintained at lowest pressure for recovery of remaining compounds.

On the basis of literature reported by various researchers involving $SCCO_2$ fractional separation of a mixture extract into heavy and lighter fractions along with insights gained from the preliminary trials, higher set of operating pressure was chosen for separator 1 (225, 200, 175 and 150 bar) and lower pressure for separator 2 (60, 50, 40, and 30 bar). An experimental set of operating pressure combination for both separators was chosen as per **Fig. 2.5** for determining best separation conditions for maximum concentration of major actives in oleoresin (non-volatile fractions) to be

recovered in separator 1 and recovery of maximum volatile fractions in separator 2. All the set of experiments for extractions coupled with fractionation were conducted at 40 ^{o}C.

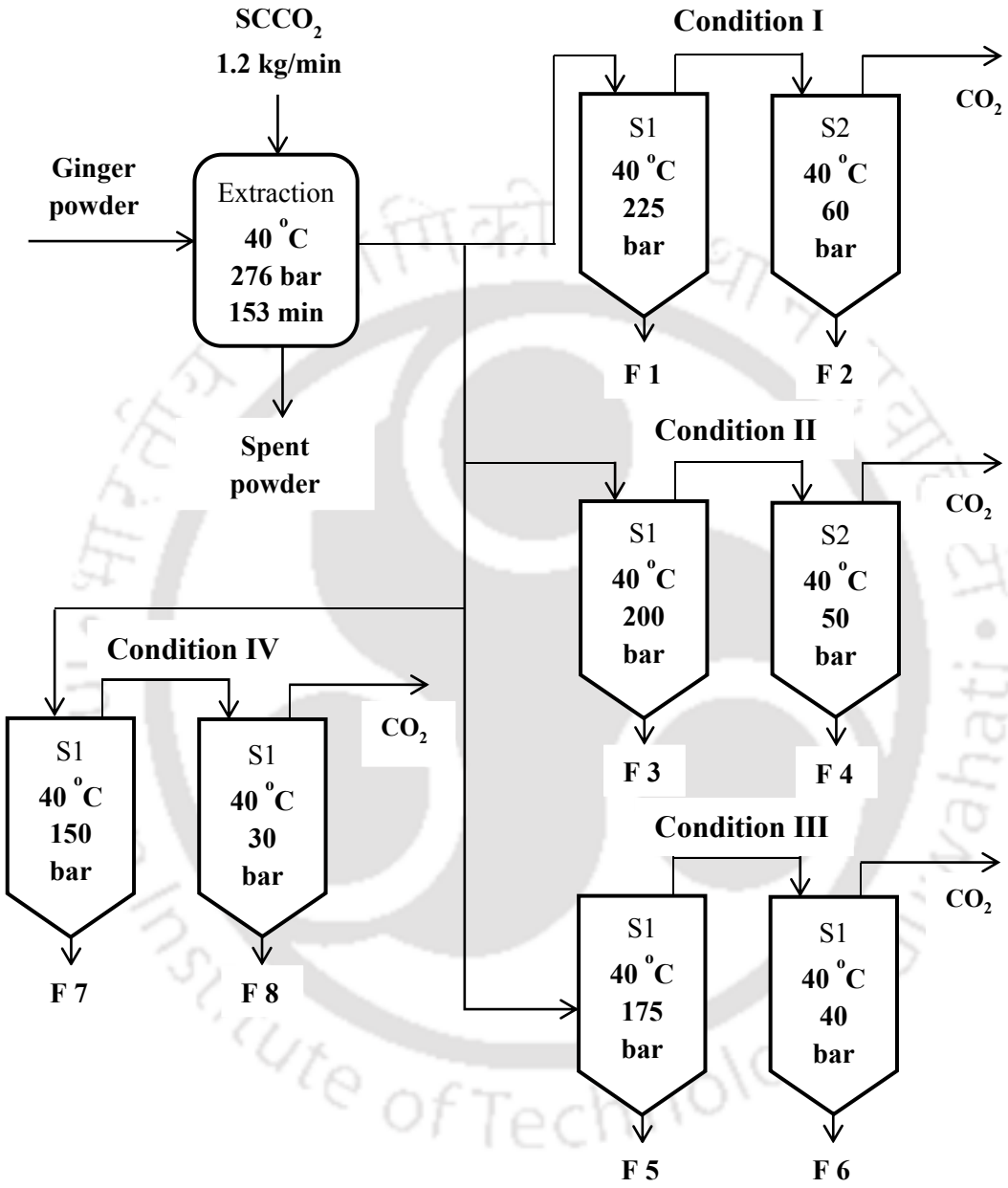


Fig. 2.5: Flow chart of extraction and online fractionation process of ginger powder

2.6 Carrier design experiments for separated gingerols enriched oleoresin

2.6.1 Excipients used

Various pharmaceutical grade excipients with bioenhancer properties used in the study were received *ex-gratis* as detailed in **Table 2.3** and used without further treatment.

TH-2388_134107006

2.6.2 Solubility studies

The equilibrium solubility of ginger actives present in ginger oleoresin (GO) in various excipients (**Table 2.3**) was determined by using the shake flask method (Date and Nagarsenker, 2007).

Table 2.3: Selected surfactants, co-surfactants and oily vehicles for the study

Sr. No.	Type	Chemical name	Commercial Name	Manufacturer
1.	Surfactant	Lauroyl polyoxyl-32 glycerides	Gelucire 44/14	Gatteffose, France
2.	Surfactant	Polyoxyethylene (20) sorbitan monooleate	Kolliphor T 80	BASF, Germany
3.	Surfactant	Macrogolglycerolricinoleate	Kolliphor RH EL	BASF, Germany
4.	Surfactant	Macrogol glycerolhydroxystearate	Kolliphor RH 40	BASF, Germany
5.	Surfactant	Polyoxyl 15 Hydroxystearate	Kolliphor RH 15	BASF, Germany
6.	Co-surfactant	Propylene Glycol (PG)	Kolliphor PG	BASF, Germany
8.	Co-surfactant	Poly Ethylene Glycol 400 (PEG 400)	Kolliphor PEG 400	BASF, Germany
9.	Co-surfactant	Diethylene Glycol Monoethyl Ether	Transcutol HP	Gatteffose, France
10.	Oily Vehicle	Propylene Glycol Monocaprylate	Capryol 90	Gatteffose, France
11.	Oily Vehicle	Propylene Glycol Monolaurate	Lauroglycol 90	Gatteffose, France
12.	Oily Vehicle	Medium-chain Triglycerides	Labrafac Lipophile	Gatteffose, France

Briefly, an excess amount of GO was added to each excipient after gentle heating, and the mixture was vortexed for 10 min, followed by sonication for 15 min in order to facilitate proper mixing. These were then stirred for 48 h at room temperature in an incubator shaker (Remi, Mumbai, India). All mixtures were centrifuged at 5500 × *g* for 10 min and filtered using membrane filter (0.2 µ, 33 mm, Axiva Life sciences, Mumbai, India). The gingerols (total of 6, 8, 10 gingerols, and 6-shogaol) in the filtrate were quantified by HPLC method.

2.6.3 Preliminary screening of excipients

Screening for best surfactant, co-surfactant, and oily vehicle among various excipients selected for the study was done based on their emulsification ability with GO by turbidimetric method (Date and Nagarsenker, 2007). Briefly, GO, and selected excipient was gently heated at 60 ºC, and 300 mg of each were mixed in a vial. 50 mg of this mixture was diluted to 50 mL with double distilled water in a stoppered flask. Ease of emulsion formation was judged by the number of flask inversion required to yield homogeneous emulsion and observed visually for relative turbidity. The percentage transmittance of the emulsions was measured after keeping them undisturbed for 2 h at 638.2 nm by UV spectrophotometer (Model: U-5100, Hitachi, Japan) against double distilled water as blank.

2.6.4 Construction of ternary phase diagrams

A series of ternary mixtures were prepared by varying surfactant, co-surfactant, and GO percentage based on recommendations for spontaneously emulsifying systems, as stated by Pouton (2000). Each mixture was gently titrated with double distilled water in a glass beaker with 1 × *g* rotation at room temperature. The tendency of the ternary mixture for spontaneous emulsification was assessed visually using the grading criteria as mentioned by (Khoo et al., 1998; Shafiq et al., 2007). If a clear and transparent dispersed system formed rapidly, it represented the best efficiently formed self-emulsification region. If a slightly turbid (slightly opaque or coarse dispersion) emulsion was formed within 2 min, it was still considered to have met the criterion for self-emulsification. However, if a dull, yellowish-white emulsion with an opaque appearance (i.e., a formulation exhibiting either poor or minimal emulsification with coarse droplets floating on the surface or complete phase separation) was formed slowly (i.e., longer than 2 min), the composition for this formulation was not considered to be in self-emulsification zone.

2.6.5 Formulation optimization by D-optimal mixture design

The D-optimal mixture is most suited for drug formulation design problems where all the components to be mixed have the same unit and variation in their proportions becomes very critical in the desired outcomes (Pickardt et al., 2009). Design Expert (7.0.0) software was utilized to optimize the best formulation from the ternary diagram showing the largest desirable area of transparent emulsion. The design consisted

TH-2388_13407006

of 5 model points and 5 points for lack of fit test. Surfactant (A), co-surfactant (B), and GO mixture (C) were independent variables, and their range was selected from the ternary diagram having maximum desired area. The design suggested 16 experiments for studying the effect of varying formulation mixture on percentage transmittance (Y_1) and globule size (Y_2). The objective for optimization of formulations was maximum percentage transmittance and size of globule. The optimum composition was predicted using the desirability function (DF) utility.

2.6.6 Characterization of prepared formulations

Globule size, polydispersity index, Zeta potential, viscosity

The mean globule size, polydispersity index (P.I.) zeta potential and viscosity of the formulations was determined by Photon Correlation Spectroscopy (Model: Delsa Nano C; Make: M/s Beckman Coulter, Switzerland). All formulations were appropriately diluted to ensure that the light scattering intensity was within the sensitivity range of the instrument. The resultant emulsions were also allowed to stand for six h at room temperature to assess dilution stability.

Dilution robustness

The prepared ginger formulation was diluted to 50, 100 and 1000 times with double distilled water. The diluted emulsion was stored for 48 h and observed for any signs of phase separation or precipitation.

In vitro release study

The in vitro release study was conducted as per the method of US Pharmacopeia at 37^{o}C, 1×*g* rotation at three different pH mediums: 1.2, 7, and 7.4 as described by (Date and Nagarsenker, 2007). Aliquots were withdrawn every 15 min, and the dissolution medium was replaced with equivalent quantity of new solution. The amount of gingerols dissolved in the aliquots was quantified by HPLC. A pH meter (Model: Eutech pH 700, Make: Thermo Fisher Scientific India Limited, Mumbai) was used to quantify the pH.

Stability study

The short term stability of the dispersion made from optimized formulation was assessed for globule size and zeta potential for 10-day storage. For long term stability,

the anhydrous optimized formulation was stored for 90 days at 40°C and 75% relative humidity and assessed for gingerols content along with globule size and zeta potential of the dispersion prepared from it.

2.7 Gingerols infused candied mango development studies

2.7.1 Raw materials

Mango fruits (*Mangifera indica* L.) of *Fazli* cultivar with uniform maturity and quality in semi-ripe condition (having shearing strength around 4.2 Nm^{-1}) were used as raw material.

2.7.2 Sample preparation

The fleshy part of fruit was cut into slices of 10 mm thickness with the help of an in-house made die. These were steam blanched at normal pressure for 5 min and subsequently cooled to room temperature. The surface moisture was blotted with tissue paper, and slices were kept in refrigerated condition for further treatments.

2.7.3 Optimization of gingerols solution strength for infusion studies

Water dispersions of optimized formulations were prepared in a range of concentrations 0.1-10 wt %, and mango slices were treated in gingerols dispersions having different gingerols strength, to facilitate gingerols infusion into mango tissues as per the industrial candying process presented in the flow chart (**Fig. 2.6**). Subsequently, the infused slices were candied in sugar solutions of 30-70 °Brix and dried in a vacuum oven till 13 % moisture content. Quality of candied product (prepared from different infusion treatments) in terms of sensory attributes was measured on a 10-point hedonic scale method by a trained panel of 10 judges.

2.7.4 Optimization of osmotic treatment for gingerols infusion and candying process

For infusion treatments, sugar solutions of different concentrations (0, 7.5, 15 and 25 °Brix) were prepared with optimized gingerols solution. Blanched mango slices were immersed for 24 h in each solution. The ratio of slices to the osmotic solution was maintained at 1:5. Samples were withdrawn every hour, rinsed with running water, blotted with tissue paper and weight. Dry matter and moisture content were determined by hot air oven method (AOAC No. 934.6). After infusion treatment, the slices were

TH-2388_1340307006

subjected to the candying process in sucrose solutions (30, 40, 50, 60 and 70 °Brix), made from the spent gingerols dispersion.

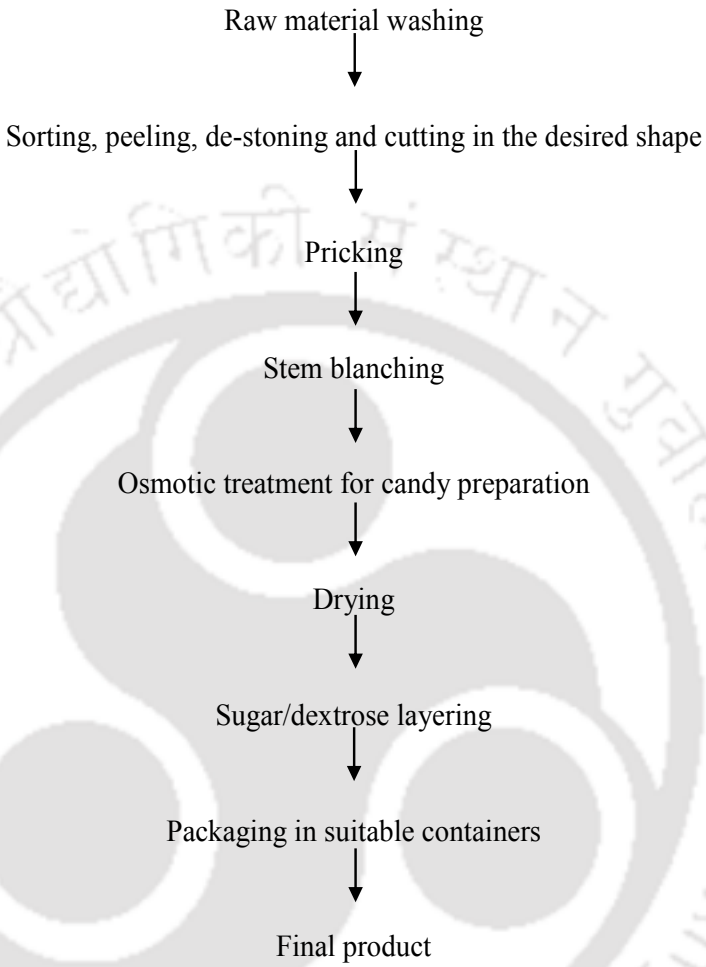


Fig. 2.6: Industrial process of mango candy production

For both bench and commercial-scale experiments, the slices were first treated in 30 °Brix solution for 24 h, manually taken out with the help of a strainer and dipped in next solution and the process repeated till 70 °Brix.

2.7.5 Data fitting

Change in weight, dry matter, and moisture content were recorded for every hour of each osmotic treatment. This was used for calculating weight loss, solid content and moisture content as per following expressions:

Calculations for water loss and solid gain

During the process of osmotic dehydration of mango pieces, the loss of weight can be calculated using the expression,

$$Weight\ loss\ =\ Solid\ gain\ +\ Water\ loss \tag{2.14}$$

At any time of this process, the solid (kg/kg) and moisture content (kg/kg) can also be calculated using the following expressions:

$$Solid\ content\ (dry\ wt.\ basis)\ =\ \frac{Solid\ content\ present\ initially\ +\ Solid\ gain}{Solid\ content\ present\ initially} \tag{2.15}$$

$$Moisture\ content\ (dry\ wt.\ basis)\ =\ \frac{Moisture\ content\ initially\ -\ Water\ loss}{Solid\ content\ present\ initially} \tag{2.16}$$

Calculations for solids, moisture, and gingerols equilibrium contents, mass transfer and diffusion coefficients

In order to understand the mass transfer characteristics for the candying as well as gingerols infusion process, change in weight, dry matter, and moisture content as well as amount of gingerols infused into mango slices was recorded for every hour of each osmotic treatment. The data of water loss, gingerols and solid gain calculated above was modelled using Fick's second law of diffusion for calculating diffusion coefficients by slope method (Perry and Green, 1999; Rastogi et al., 2002). According to Fick's law, the diffusion equation for unsteady is as follows (Crank, 1975):

$$\frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial x^2} \tag{2.17}$$

Where, C, t, D_e, and x are concentration, time, diffusion coefficient, and diffusion path.

Considering diffusion from an infinite flat plate having thickness 2a, being subjected to osmotic dehydration from both the major faces with the following assumptions:

1. Initial moisture distribution being uniform
2. External resistance to mass transfer to be negligible
3. Negligible shrinkage of the flat plate during osmotic dehydration; along with the following initial and boundary conditions:
    a. $C = C_o$ at $t = 0$; $-l < x < +l$
    b. $C = C_l$ at $t > 0$; $x = l$

The solution of Equation (2.17) hence results into the following well-known

Equation (2.18) for solid and moisture transfer (Crank, 1975; Rastogi et al., 2002):

$$X_i = \frac{(x_{ti} - x_{\infty i})}{(x_{oi} - x_{\infty i})} = \sum_{n=1}^{\infty} C_n \exp\left[-D_{ei}\ t\ q_n^{\,2}\left(\frac{1}{a^2}\right)\right] \tag{2.18}$$

Expressing the Fourier number as $D_{ei}t/a^2$ for solid or moisture and substituting this in above Equation gives the following equation:

$$X_i = \frac{(x_{ti} - x_{\infty i})}{(x_{oi} - x_{\infty i})} = \sum_{n=1}^{\infty} C_n \exp\left[-F_{oi} q_n^{\,2}\right] \tag{2.19}$$

Where X_i is the dimensionless ratio of solid or moisture; o, t and ∞ express the concentrations initially, at any time and at equilibrium; '*i*' becomes '*s*' for solid, '*m*' for moisture and '*g*' for gingerols contents respectively; D_{ei} being the effective diffusion coefficient; and the value of C_n can be calculated by $2\alpha(1+\alpha)/(1+\alpha+\alpha^2 q_n^2)$ where, q_n's are the non-zero positive roots of the equation $\tan q_n = -\alpha q_n$. α being the ratio of volume of solution to each piece.

Equation (2.19) was expressed graphically by plotting $\log (X_i)$ against Fourier number (F_{oi}). The slope of this graph was a theoretical diffusion line [$d(\log X_i)/dF_{oi}$] whose value was found to be 1.075 (Rastogi et al., 2002). (Appendix 4)

Considering the equilibrium approach to mass transfer, the following equation for solid or moisture mass transfer can be written (Rastogi et al., 2002):

$$-\frac{dx_i}{dt} = k_i (x_{ti} - x_{\infty i}) \tag{2.20}$$

Where k_i is the moisture mass transfer coefficients. Integration of Eq. (2.17), with the appropriate initial condition, resulted in the following equation:

$$\ln \frac{(x_{ti} - x_{\infty i})}{(x_{oi} - x_{\infty i})} = \ln X_i = -k_i t \tag{2.21}$$

The rate of change of moisture, solids, and gingerols content was plotted against its average moisture, solids, and gingerols content, respectively, for each treatment of infusion as well as the candying process (Appendix 4). The moisture (k_w), solid (k_s) and gingerols (k_g) mass transfer coefficients, as well as equilibrium moisture ($x_{\infty w}$), solid ($x_{\infty s}$) and gingerols ($x_{\infty g}$) contents were inferred from the slope and intercept of these plots. The slope was calculated from this equilibrium data as follows:

$$\frac{d}{dt}(\log X_i) = -\frac{k_i}{2.3025} \tag{2.22}$$

Considering an infinite flat plate configuration, D_{ei} values were estimated from the following equation (Perry and Green, 1999; Rastogi et al., 2002):

$$D_{ei} = \left[\frac{d(\log X_i)/dt}{d(\log X_i)/dF_{oi}} \right] \cdot a^2 \tag{2.23}$$

The values of solid diffusion coefficients (D_{es}), moisture diffusion coefficients (D_{em}), and gingerols diffusion coefficients (D_{eg}) into mango slices were calculated for the candying process using Eq. (2.23).

2.7.6 Drying of candied mango slices

Candied slices were dried in a commercial scale tray and a vacuum dryer (M/s Bajaj Process Pack, Sahibabad, India) to a final moisture content of 13.5% and 0.4-0.45 water activity. Moisture content recorded at every 30 min was used for calculating drying rates (k) and moisture diffusivities (D_e) as per methods of Dermesonlouoglou et al., (2018) and Madamba et al., (1996), respectively.

2.7.7 Product Characterization

Sample preparation for estimation of gingerols

Slices were mixed with 5 mL ethanol and ground in mortar and pestle and the process repeated five times for complete extraction. The supernatant collected in each step was pooled and centrifuged at 2500 × *g* for 30 min at 5 °C. The resulting supernatant was removed and used for estimation of gingerols (the sum total of 6, 8, 10 gingerols and 6-shogaol).

β-carotene

Extraction of β-carotene from the sample was carried out in a similar fashion using 10 mL of n-hexane and ethanol mixture (1:1[v/v]) in mortar and pestle. Hexane was used in combination with ethanol to remove hydrophobic β-carotene from the sample paste. Only non-polar solvent (hexane) was of little use because of its limited penetration through the hydrophilic mass (because of moisture present in candied mango samples) surrounded by β-carotene pigment. Hence hexane mixed with a polar solvent (ethanol) facilitated complete extraction of β-carotene from samples of candied mango. The supernatant from mortar and pestle was filtered with Whatman No. 4 filter paper, and successive extractions were done until the sample paste turned colorless. The pooled

TH-2388_1352207006

extract was washed with an equal quantity of water and then vortexed to separate the hexane from the aqueous phase. The combined hexane extract was used for estimation of β-carotene with the help of standard β-carotene calibration plot (Appendix 5) using spectrophotometer at 450 nm as per the method reported by (Ndawula et al., 2004).

Vitamin C

Vitamin C was quantified using the method as described by Ranganna (1986). Mango candy sample (10 g) was ground in 100 mL of 3 % metaphosphoric acid. This mixture was centrifuged at 5500 × *g* for 30 min at 10 °C, and the supernatant was filtered. Vitamin C content of this filtrate was calculated by titrating a dye solution of 2, 6-dichlorophenolindophenol.

Color characteristics

The color properties in terms of *L* (black to white), *a* (green to redness) and *b* (blue to yellow), were measured using a handheld colorimeter (MiniScan EZ 4000S, M/s. Hunter lab, USA). The total color difference (*ΔE*) and browning index (BI) were calculated from this data as per the method described by (Chakraborty et al., 2016; Kaushik et al., 2014) as per following formulae:

$$\Delta E = \sqrt{(L_n - L_0)^2 + (a_n - a_0)^2 + (b_n - b_0)^2} \quad (2.24)$$

$$BI = \frac{180 \times (a + 1.75L)}{5.645L + a - 3.012b} \quad (2.25)$$

Where, superscript n corresponds to color value at nth day and 0 belongs to day 0

Sensory scores

The sensory scores of the final product were determined by 9-point hedonic scale method (Ranganna, 1986). The sensory panel comprised of 10 trained judges and the product was evaluated between a score value (*S*) of 1 to 9 where 1 was the least score and 9 being the best for the following chosen parameters: appearance, crunchiness, mouthfeel, after taste, aroma, sweetness, sourness, and pungency. The trained panel defined the choice of each parameter for our specific product and weightage (*W*) for each parameter was assessed between a score of 1 to 5, where 1 being least important and 5

being most important. Overall sensory score (*OSS*) was calculated as per following expression in which *n* denoted number of chosen parameters.

$$OSS = \frac{1}{n}\left(\frac{\sum S \times W}{\sum W}\right) \quad (2.26)$$

Shearing property

A texture analyzer (Model-CT3, M/s Ametek Brookfield, USA) with a 4.5 kg force load cell having a cylindrical probe connected with a Warner blade was used to shear the candied sample into two pieces at 2.0 mm s^{-1} test speed. The test type was compression. An average value of four measurements for each sample was reported as shear force (N m^{-1}).

2.8 Storage studies

2.8.1 Packaging and storage conditions

The packaging, storage, and sampling conditions were chosen based on the recommendation for dehydrated fruit-based products with similar moisture content profiles (Taoukis et al., 1997). Commercially available stand-up zipper pouches (outer dimensions: 11″ × 6″ × 2″ [bottom gusset], having 500 g capacity), were chosen based on their suitability for long term storage of dehydrated food products. Pouches with two different materials having high barrier properties were selected for the storage stability study.

The Aluminium metal-based multi-layered material with following specifications: 12 µm PET (polyethylene terephthalate) + 9 µm Aluminium foil + 12 µm PET + 83 µm polypropylene, was designated as MET. Whereas, ethylene-vinyl alcohol coextruded with PET having following specifications: 12 µm PET + 100 µm ethylene-vinyl alcohol was designated as EVOH. Pouches with selected materials and specifications are widely used in commercial packaging of fruit-based dehydrated products for their high barrier to oxygen and moisture (Chakraborty et al., 2016).

A suitable amount of gingerols infused candied mango, and non-infused candied mango (control) were kept in both MET and EVOH pouch, sealed and stored at three different temperatures 25, 35 and 45 °C for 120 days.

TH-2388_13407006

2.8.2 Browning characteristics

It is a widely known prior art in the commercial-scale manufacturing sector as well as through the results of various researchers that the most limiting factor for the shelf life of osmotically dehydrated fruits having low moisture content and water activity is browning due to non-enzymatic reactions (Taoukis et al., 1997). Hence, the non-enzymatic browning in the candied mango samples was estimated spectrophotometrically as per the method of (Kaushik et al., 2018). The absorbance of the ethanolic extract of the samples was measured at 420 nm using ethanol as blank and designated as the browning index (BI).

2.8.3 Shelf life estimation

Univariate kinetic modelling based accelerated shelf-life testing (ASLT)

The data on quality parameters of the product *viz.* gingerols, TPC, AC, vitamin C, BI, β-carotene and sensory scores were fitted to zero-order (Eq. 2.27) and first-order (Eq. 2.28) models. Arrhenius equation (Eq. 2.29) was used to calculate the activation energy (E_a)

$$A_t = A_o - kt \tag{2.27}$$

$$A_t = A_o e^{-kt} \tag{2.28}$$

$$k = k_o \times e^{-(Ea/RT)} \tag{2.29}$$

where, A_t is the concentration of chosen quality parameter at any time t and A_o at starting of experiments (t = 0), k being the kinetic rate constant, k_o pre-exponential factor, R is universal gas constant, and T is the storage temperature. Univariate (conventional) kinetics based shelf life of the product was calculated for each of the studied quality parameters using its degradation rate (k) up to threshold value. Based on literature values as well as the judgment of trained sensory panel, the threshold values decided were 50 % retention of functional parameters, sensory scores ≥ 5 and 2 times increase in BI values (Dermesonlouoglou et al., 2018; Kaushik et al., 2018).

Multivariate accelerated shelf-life testing (MASLT)

The univariate kinetic based approach gives different cut-off values for each individual parameter, and hence prediction of a safe shelf storage period for an industrial product becomes a difficult task. The complexity is increased when the shelf-life

predictions have to be made among many parameters critically responsible for simultaneously inducing spoilage reactions. Hence it was also important to understand how quality parameters affect each other during the course of the accelerated shelf-life testing period.

For this purpose, chemometrics based tool, principle component analysis (PCA) was used to reduce the multidimensionality of the entire shelf-life data (Upadhyay et al., 2017). Data for all quality attributes estimated for each temperature were arranged sequentially in a matrix, and PCA was performed to extract principal components (PCs) as per the procedure described by Derossi et al., (2016). The score values of the extracted PCs were plotted with respect to time, and the kinetic fitting of this data was performed as per previous section. A new rate constant, multivariate rate ($k_{multivariate}$) was calculated for each temperature (T), and a multivariate acceleration factor ($\alpha_{multivariate}$), analogous to Q_{10} values were also calculated from $k_{multivariate}$ values following the method described by (Sehwag et al., 2018) as follows:

$$\alpha^{T}_{multivariate} = \frac{k^{T+\delta T}_{multivariate}}{k^{T}_{multivariate}} \tag{2.30}$$

Following this, multivariate activation energy ($E_{a,\ multivariate}$) was also estimated. Consequently, a multivariate threshold criterion ($t_{multivariate}$) was estimated which was a product of matrix containing threshold values for each quality parameters and a matrix containing loading values for each of extracted PC1 scores. Finally, shelf life based on collated effects of all the quality parameters was calculated based on this new multivariate threshold criterion.

2.9 Data analysis

Estimation of all the parameters was conducted in triplicate and values reported as average along with their standard deviations. Duncan's multiple range tests were conducted at 5% significance level using SPSS software version 20, IBM Corporation (New York, USA). Pearson's correlation coefficient (r) and PCA was carried out using OriginPro 9.0 software, Origin Lab Corporation, Northampton (Massachusetts, USA). An example of error analysis calculation has been presented in Appendix 6.

TH-2388_136107006

Chapter 3

Identification of best ginger varieties from northeast India from the perspective of antioxidant and nutritional potential

Work published at:

Shukla, A., Goud, V.V., Das, C., 2019. Antioxidant potential and nutritional compositions of selected ginger varieties found in northeast India. *Industrial Crops and Products*, *128,* 167-176.

TH-2386_136107006

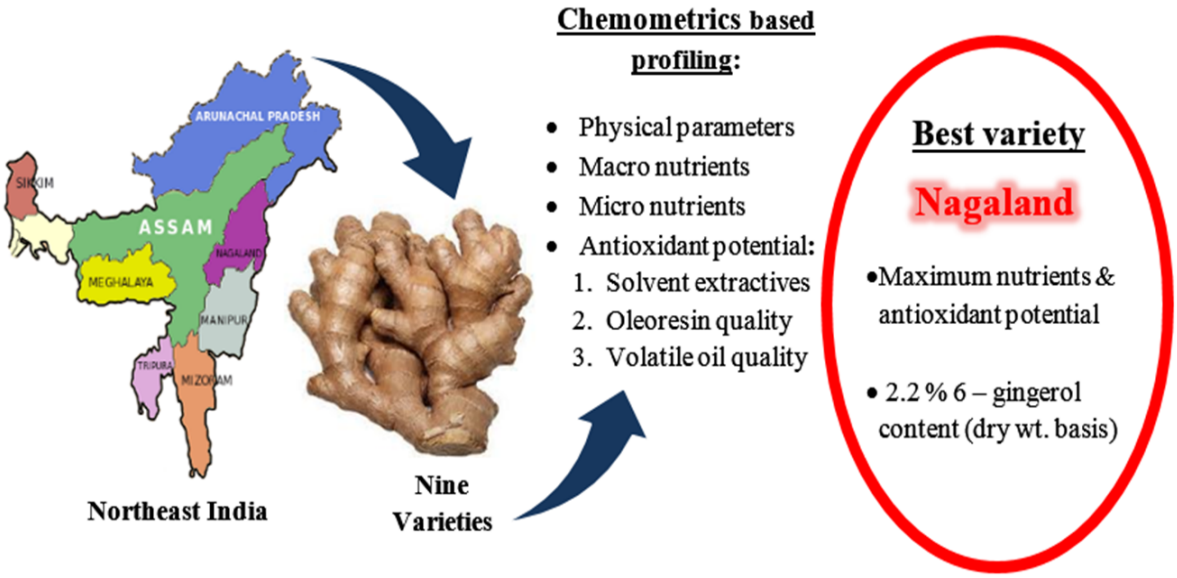
TH-2386_136107006

3.1 Background

Northeast India is one of the largest biodiversity hotspots regions of the world. The unique geographical conditions enjoyed by this region is responsible for producing one of the finest quality agricultural commodities in the global market. Ginger is one of the main cash crops of this region, hence the ginger varieties found in this region could potentially be one of the best varieties of the world. Unfortunately, very few studies are available to support this hypothesis. Hence, in this respect an attempt has been made to carry out systematic physico-chemical characterization of different ginger varieties from diverse regions of northeast India. The database presented, could serve as a readymade tool for selecting and screening ginger varieties for specific industrial applications.

3.2 Overview

In the present chapter, a systematic profiling of nine ginger cultivars collected from different states of northeast India has been presented. The screening of varieties for various industrial applications has been attempted based on multivariate chemometric tools and correlation analysis. The detailed database of forty-one parameters presented pertains to the physical, morphological and nutritional properties, essential minerals, antioxidant properties of extractives, active ingredients of oleoresin and volatile oils. The relative abundance of mineral contents followed a trend of K > Na > Mg > Ca > Fe > P > Mn > Zn > Cr > Ni > Cu for all the varieties. Ethanol proved to be the best solvent for extracting maximum phenolic (0.96 mg g^{-1}) and flavonoids (1.52 mg g^{-1}) content from dried rhizomes. Sungro-sung variety from Nagaland was found to contain surprisingly high 6-gingerol content (2.2 wt % dry basis) which has not been reported till date. Chemometric tools clearly segregated Sungro-sung followed by Arunachal Pradesh local varieties as the best in terms of overall antioxidant as well as nutritive potential. The correlation study within all the parameters revealed that the average ginger rhizome size inversely depended on the major bioactive constituents of oleoresin and volatile oils of all the varieties. This single parameter can potentially be used as a rapid inexpensive non-destructive method in developing countries for screening the ginger varieties for commercial production of volatile oil and oleoresin.



Graphical abstract of the work presented in this chapter

Indian Institute of Technology Guwahati

3.3 Results and discussion

All the samples of 9 ginger varieties collected from different locations of northeast India were coded and discussed throughout the chapter as per their sample codes presented in **Table 3.1**.

Table 3.1: Sample codes of different ginger varieties

Sample Code	Variety Name	Origin
1	*Gangtok Local*	Sikkim
2	*Imphal Local*	Manipur
3	*Arunachal Pradesh Local*	Arunachal Pradesh
4	*Thingpui*	Mizoram
5	*Sungro-sung*	Nagaland
6	*Karbi Anglong*	Assam
7	*Haem*	Meghalaya
8	*Malang*	Meghalaya
9	*Noangphu*	Tripura

3.4 Physical parameters

Physical parameters of all the nine varieties are presented in **Table 3.2**. The range of average size (52.9 – 122.3 mm) and weight (76.2 – 250.8 g) of the collected samples were found to be on the higher side compared to the reported values of 24 – 33 mm and 45.7 – 60.9 g, respectively (Hazarika and Kakoti, 2013), implying the large rhizomes size. The relationship between average size and weight was intuitively found to have a positive strong correlation, presented by Pearson's correlation coefficient of r = 0.99 for all the varieties as presented in **Table 3.3**. However, the edible index range of 83.2 to 93.5 % of whole rhizome, was slightly less than 85.7 to 97.6 % of literature values (Hazarika and Kakoti, 2013) due to the higher content of inedible adventitious roots which ultimately reduced its edible index. The moisture content of the rhizomes (71.6 – 90.5 %) was comparable to the major ginger varieties of the world (Govindarajan and Connell, 1983).

The water retention capacity (WRC) and swelling capacity (SC) of the collected nine varieties have been estimated between 3.5 to 8.1 and 4.8 to 10 g H_2O g^{-1} dry powder,

respectively and are found positively correlated (r = 0.85). The observed high values of moisture content, WRC and SC, average size and weight for all the varieties are ideal for industrial processing of a variety of products like paste, juice, shreds, slices (for preserve production) (Raghavendra et al., 2004). These values become critical in a wide range of other applications pertaining to design and product development of speciality food and pharmaceutical products (Ismail and Lingamallu, 2012; Kubra and Rao, 2012).

3.5 Nutritional parameters

3.5.1 Macronutrients

The macronutrient parameters of the collected varieties were found to vary widely with each other as shown in **Table 3.4**. The crude fiber content has been found to vary from 8.9 wt % (*Haem*) to 17.9 wt % (*Arunachal Pradesh local*) which was similar to the published values of 8 – 20% for major commercial varieties of the world.

A negative correlation (r = -0.67) was found between moisture and crude fiber contents of all the varieties (**Table 3.2**). A similar relationship was reported for all the major fiber containing root crops of the world (Parthasarathy et al., 2008). This inverse correlation gives important insights on screening the ginger varieties for specific processing applications involving fresh rhizomes having high moisture and low fiber content and also in selecting appropriate drying mechanism for the same (Ismail and Lingamallu, 2012). *Thingpui* variety recorded the lowest total ash content (4.6 wt %), while *Sungro-sung* variety had the highest value (13.1 wt %). This parameter corresponds to good processing and handling ability for selected ginger varieties. The observed variation in total ash content was in good agreement with the reported range of high-value commercial varieties (5.1 – 9.3 wt %) of the world (Govindarajan and Connell, 1983).

The crude protein content varied significantly ($p<0.5$) from 7.6 wt % (*Thingpui*) to 17 wt % (*Karbi Anglong*), which was comparable to the reported values of 10.3 – 15 wt %. The contents of starch (0.11 – 0.87 wt %), total sugars (0.31 – 1.43 wt %) and reducing sugars (0.2 – 0.91 wt %) in the current study were also comparable to the published data of 1.15 wt % and 0.2 wt % of the total and reducing sugars, respectively (Govindarajan and Connell, 1983). The fat content varied from 0.94 (*Gangtok local*) to 2.69 wt % (*Sungro-sung*) and showed negative correlation with the average size (r = -0.73) and weight (r = -0.7) for all the varieties (**Table 3.2**).

TH-2388_136107006

TH-2386_136107006

Table 3.2: Physical parameters of different ginger varieties

Sample Code	Physical parameters					
	Avg. size of rhizome[1], mm	Avg. wt. of rhizome[1], g	Edible index[1], % weight	Moisture content[1], %	WRC[2], g H_2O/g dry powder	SC[2], ml H_2O/g dry powder
1	122.3 ± 5.5^{g}	250.8 ± 8^{f}	90.2 ± 0.3^{d}	88 ± 1.1^{e}	4.3 ± 0.2^{c}	6.8 ± 0.1^{c}
2	59 ± 0.5^{b}	80.8 ± 3.2^{a}	86.2 ± 1.2^{b}	72.4 ± 0.3^{a}	4 ± 0.1^{b}	5 ± 0.2^{a}
3	85.3 ± 1.6^{e}	158.2 ± 5.7^{d}	93.5 ± 0.6^{f}	72.6 ± 1.4^{a}	3.5 ± 0.1^{a}	4.8 ± 0.2^{a}
4	66.1 ± 2^{c}	102.6 ± 4.8^{b}	87 ± 1.8^{b}	71.6 ± 0.9^{a}	4 ± 0.1^{b}	7 ± 0.3^{c}
5	52.9 ± 1.7^{a}	76.2 ± 2.3^{a}	88.6 ± 1.3^{c}	81.7 ± 1^{c}	3.9 ± 0.1^{b}	7 ± 0.3^{c}
6	117.5 ± 3.8^{f}	240.7 ± 10.8^{e}	94.4 ± 0.4^{f}	85.2 ± 1.9^{d}	7.2 ± 0.1^{d}	8 ± 0.3^{e}
7	66.2 ± 2^{c}	101.4 ± 1.1^{b}	88.8 ± 0.8^{cd}	90.5 ± 1.8^{f}	4.7 ± 0.1^{c}	7.6 ± 0.3^{d}
8	70.6 ± 1.8^{d}	114.9 ± 5.9^{c}	92 ± 0.4^{e}	76.8 ± 1.8^{b}	3.7 ± 0.1^{a}	6 ± 0.2^{b}
9	64.4 ± 1.9^{c}	98.9 ± 1.4^{b}	83.2 ± 3.6^{a}	78.8 ± 1.8^{b}	8.1 ± 0.3^{e}	10 ± 0.3^{f}

1: parameters determined on a fresh weight basis, 2: parameters determined on a dried weight basis, subscript letters within the same column represent significant differences at $p<0.05$

TH-2388_136207006

Table 3.3: Pearson correlation coefficients (r) between physical and macronutrient parameters of different ginger varieties

Parameters	Crude fiber	Crude protein	Starch	Total Sugars	Reducing sugars	Total Ash	Carbohydrate	Fat	Avg. size	Avg. weight	Edible Index	Moisture Content	WRC
Crude protein	-0.26												
Starch	-0.01	-0.3											
Total sugars	0.06	-0.35	0.99*										
Reducing sugars	-0.05	-0.26	0.99*	0.98*									
Total Ash	0.18	0.48	-0.32	-0.37	-0.37								
Carbohydrate	-0.24	0.27	-0.32	-0.28	-0.24	-0.29							
Fat	0.57	0.23	-0.25	-0.2	-0.29	0.49	0.12						
Avg. size	-0.42	0.12	0.17	0.07	0.22	0.08	-0.18	-0.73*					
Avg. weight	-0.39	0.14	0.16	0.06	0.2	0.12	-0.18	-0.7*	0.99*				
Edible Index	-0.12	0.47	-0.05	-0.16	0.02	0.32	0.09	-0.05	0.62	0.64			
Moisture Content	-0.67*	0.34	-0.05	-0.12	-0.05	0.46	0.06	-0.24	0.43	0.44	0.17		
WRC	-0.3	-0.05	-0.59	-0.59	-0.57	-0.13	0.16	-0.5	0.23	0.22	-0.21	0.27	
SC	-0.25	-0.19	-0.31	-0.3	-0.31	-0.1	0.17	-0.36	0.04	0.04	-0.41	0.43	0.85*

*Significant at $p < 0.05$

Table 3.4: Macronutrient parameters of different ginger varieties

Sample Code	Macronutrient parameters							
	Crude fiber[2], %	Crude protein[2], %	Starch[2], %	Total sugars[2], %	Reducing sugars[2], %	Total Ash[2], %	Carbohydrate[2], %	Fat[2], %
1	10.6 ± 0.3^{c}	12.3 ± 0.1^{d}	0.87 ± 0.01^{g}	1.43 ± 0.01^{h}	0.91 ± 0.02^{g}	10.2 ± 0.2^{e}	58.4 ± 2.2^{a}	0.94 ± 0.03^{a}
2	12.1 ± 0.2^{d}	14.3 ± 0.1^{e}	0.37 ± 0.01^{d}	0.81 ± 0.01^{e}	0.42 ± 0.01^{d}	7.8 ± 0.1^{b}	62.7 ± 3.4^{bcde}	1.83 ± 0.05^{e}
3	17.9 ± 0.4^{h}	11.3 ± 0.4^{c}	0.29 ± 0.01^{c}	0.61 ± 0.01^{c}	0.33 ± 0.01^{c}	11.9 ± 0.4^{g}	59.4 ± 2.1^{ab}	2.21 ± 0.07^{g}
4	14.8 ± 0.5^{f}	7.6 ± 0.5^{a}	0.72 ± 0.01^{f}	1.33 ± 0.01^{g}	0.81 ± 0.01^{f}	4.6 ± 0.1^{a}	64.6 ± 2.2^{cd}	1.6 ± 0.09^{d}
5	16.8 ± 0.1^{g}	17 ± 0.6^{g}	0.37 ± 0.01^{d}	0.79 ± 0.01^{d}	0.4 ± 0.01^{d}	13.1 ± 0.2^{h}	63.3 ± 2.8^{bcd}	2.69 ± 0.09^{h}
6	9.6 ± 0.6^{b}	16.8 ± 0.1^{g}	0.11 ± 0.01^{a}	0.31 ± 0.01^{a}	0.22 ± 0.01^{b}	9.2 ± 0.3^{d}	65.9 ± 2.7^{d}	1.13 ± 0.04^{b}
7	8.9 ± 0.3^{a}	12.6 ± 1.1^{d}	0.3 ± 0.01^{c}	0.61 ± 0.01^{c}	0.33 ± 0.01^{c}	10.8 ± 0.4^{f}	63.4 ± 2.4^{bcd}	2.04 ± 0.07^{f}
8	12.4 ± 0.4^{d}	15.7 ± 0.1^{f}	0.61 ± 0.01^{e}	1.02 ± 0.01^{f}	0.67 ± 0.02^{e}	8.5 ± 0.2^{c}	61.4 ± 2.1^{abc}	1.86 ± 0.06^{e}
9	14.2 ± 0.7^{e}	9.7 ± 0.1^{b}	0.18 ± 0.01^{b}	0.51 ± 0.01^{b}	0.2 ± 0.01^{a}	8.6 ± 0.3^{c}	60.6 ± 2.5^{abc}	1.43 ± 0.05^{c}

1: parameters determined on a fresh weight basis, 2: parameters determined on a dried weight basis, subscript letters within the same column represent significant differences at $p<0.05$

The varieties with high-fat content are ideal for the industrial production of lipid-based extracts, commercially used as natural ginger flavorings in food and in the formulation of pharmaceutical drugs (Kubra and Rao, 2012). The high scores of some of the nutritional parameters (fat, total ash, and fiber contents) for *Sungro-sung* variety were found to be higher than the commercial ginger varieties (Govindarajan and Connell, 1983). The rich biodiversity of northeast India houses one of the most favorable agro-climatic conditions in the world, responsible for higher nutrient uptake by plants. This fact is supported by various published reports which have quantitatively demonstrated high contents of quality markers for various fruits and vegetables endemic specifically to this region (Hazarika and Kakoti, 2013; Kiran et al., 2013; Majumder et al., 2011; Ravi Kiran et al., 2013). This possibly makes *Sungro-sung* variety the richest in nutritive characteristics among all the nine varieties.

3.5.2 Micronutrients

Among a wide range of elements absorbed by the ginger plant from soil, some of them have been reported to play a vital health beneficiating role in various day to day biochemical reactions in human cells (Pandotra et al., 2015). **Table 3.5** presents micronutrient profile of the collected ginger varieties in terms of their essential mineral compositions (expressed in ppm on a dry weight basis). These were found within a range of 365 to 837.3 for Na, 1487.7 to 2547.2 for K, 260.6 to 673.9 for Ca, 108.9 to 422.6 for P, 67.4 to 617.1 for Fe, 14.3 to 129.1 for Mn and 407.8 to 765.3 for Mg. Whereas, the range of Zn was found to be 3.6 to 7.6, 0.08 to 0.31 for Cu, 0.53 to 1.28 for Ni and 0.84 to 1.71 for Cr. The relative micronutrient contents for all the ginger varieties in *descending order* of abundance were found to be as follows: K > Na > Mg > Ca > Fe > P > Mn > Zn > Cr > Ni > Cu. Except for Na, this order was consistent with the reported data of 46 ginger varieties from northern India (Pandotra et al., 2015). A recent study on south Indian ginger variety reported similar values of Ca (884), Fe (80), Zn (9.2) and Cr (0.7) however its P (1740) and Mg (91.3) contents were substantially higher and lower, respectively than the current values (Adel and Prakash, 2010). On the contrary, Otunola et al. (2010) in their study of Nigerian ginger variety reported significantly lower values of Na (41), K (540), Mn (0.01), Mg (41), Fe (52.9) and P (101.9) contents. However, their reported values of Ca (263) and Zn (3.4) contents were found to be comparable with the present study. The contents of Na and Cu were found to highly correlate with the Ni contents in all the varieties.

TH-2388_136107006

TH-2386_136107006

Table 3.5: Micronutrient profile of different ginger varieties (ppm on a dry weight basis)

Varieties	Na	K	Ca	P	Fe	Zn	Cu	Mn	Ni	Cr	Mg
1	837.3 ± 24.7^{h}	2020.4 ± 69.1^{c}	413.8 ± 14.9^{c}	108.8 ± 25.6^{a}	176.7 ± 6.5^{d}	4.8 ± 0.1^{d}	0.17 ± 0.01^{d}	25.2 ± 0.1^{b}	0.57 ± 0.02^{a}	0.84 ± 0.03^{b}	621 ± 27.4^{d}
2	447.5 ± 19.3^{d}	1487.7 ± 41.1^{a}	396.6 ± 15.3^{c}	217.3 ± 5.6^{c}	232.7 ± 8.7^{e}	6.6 ± 0.2^{f}	0.11 ± 0.01^{bc}	107 ± 0.1^{h}	0.53 ± 0.02^{a}	1.36 ± 0.04^{e}	559.6 ± 23.1^{c}
3	365 ± 17.5^{a}	2417.6 ± 85.7^{d}	331 ± 13.4^{b}	422.6 ± 11.2^{f}	187.6 ± 5.1^{d}	7.2 ± 0.2^{g}	0.09 ± 0.01^{ab}	56.5 ± 0.1^{d}	0.55 ± 0.01^{a}	1.71 ± 0.06^{h}	407.8 ± 22.3^{a}
4	422.1 ± 13.6^{cd}	2397.8 ± 66.1^{d}	410.1 ± 16.5^{c}	166.1 ± 0.5^{b}	96.4 ± 2.9^{b}	4.1 ± 0.1^{c}	0.11 ± 0.01^{bc}	38.5^{c}	0.66 ± 0.03^{b}	1.46 ± 0.05^{f}	633.5 ± 31.2^{d}
5	455.4 ± 16.2^{e}	2547.2 ± 116.7^{e}	673.9 ± 32^{f}	275.7 ± 28.7^{d}	617.1 ± 23.3^{g}	7.2 ± 0.3^{g}	0.31 ± 0.02^{e}	58.3 ± 0.1^{e}	1.28 ± 0.04^{c}	1.15 ± 0.03^{d}	765.3 ± 31.3^{e}
6	415 ± 19.9^{bc}	1870.1 ± 81.5^{b}	344.2 ± 8.2^{b}	153.7 ± 23.9^{b}	114.5 ± 4.4^{c}	7.6 ± 0.2^{h}	0.08 ± 0.01^{a}	129.1 ± 0.1^{i}	0.57 ± 0.03^{a}	0.94 ± 0.03^{c}	500.3 ± 20.5^{b}
7	387.9 ± 14.6^{ab}	2065 ± 72.8^{c}	591.1 ± 15.6^{e}	120.2 ± 5.9^{a}	183.9 ± 6.1^{d}	6.1 ± 0.2^{e}	0.09 ± 0.01^{ab}	73.7^{g}	0.55 ± 0.01^{a}	1.30 ± 0.04^{e}	582.5 ± 26.9^{c}
8	542.7 ± 20.4^{g}	2457.5 ± 71.2^{e}	559.3 ± 24.3^{d}	213.6 ± 15.3^{c}	251 ± 12.3^{f}	3.6 ± 0.1^{b}	0.11 ± 0.01^{bc}	61.5^{f}	0.57 ± 0.02^{a}	1.59 ± 0.06^{g}	432.5 ± 16.6^{a}
9	487.3 ± 20.6^{h}	2387.1 ± 78.1^{d}	260.6 ± 8.6^{a}	327.2 ± 5.7^{e}	67.4 ± 3.3^{a}	2.9 ± 0.1^{a}	0.12 ± 0.01^{c}	14.3 ± 0.1^{a}	0.62 ± 0.03^{b}	1.57 ± 0.02^{a}	422.9 ± 17.3^{a}

subscript letters within the same column represent significant differences at $p<0.05$

Table 3.6: Pearson correlation coefficients in 11 essential micro nutrients of different ginger varieties

Element	Na	K	Ca	P	Fe	Zn	Cu	Mn	Ni	Cr
K	-0.09									
Ca	-0.07	0.15								
P	-0.4	0.45	-0.55							
Fe	-0.01	0.42	0.54	-0.11						
Zn	-0.42	0.09	-0.29	0.41	0.13					
Cu	0.91*	0.002	-0.14	-0.3	0.08	-0.26				
Mn	-0.2	-0.18	-0.21	0.47	0.03	0.69*	-0.12			
Ni	0.93*	-0.1	-0.05	-0.42	-0.08	-0.54	0.93*	-0.32		
Cr	-0.14	-0.01	0.5	0.05	0.13	-0.02	-0.24	0.4	-0.11	
Mg	0.58	-0.24	0.35	-0.68*	0.33	-0.33	0.73*	-0.23	0.7*	-0.1

*Significant at $p < 0.05$

This was shown by the correlation coefficients of 0.91 and 0.93, respectively, as presented in **Table 3.6.** Similarly, Cu was related with Ni and Mg, Zn with Mn and Mg with Ni contents. However, P was negatively correlated with Mg contents (r = -0.68) for all the ginger varieties. These observed relationships were consistent with that of the reported elemental relationships in a study of ginger varieties from north India. However, the P content was not reported and no relationship was found within Na and Ni contents for all the varieties (Pandotra et al., 2015). It is noteworthy that *Sungro-sung* variety was found to have the highest contents each for K, Ca, Fe, Mg, Ni, and Cu. These values were also supported by its highest total ash content of 13.1 % (**Table 3.2**). Thus, it can be concluded that in addition to its good nutritive profile, an abundance of these elements in *Sungro-sung* ginger variety makes it most useful to people prone to specific micronutrient deficiency ailments such as blood and bone diseases like anemia, osteoporosis and low hemoglobin. In addition to this, it also serves as an excellent anti-allergen, along with digestion and metabolism enhancer (Ismail and Lingamallu, 2012; Parthasarathy et al., 2008; Shukla and Singh, 2007; Srinivasan, 2017).

3.6 Antioxidant parameters

3.6.1 Solvent extractives

Ginger is most known the world over for its excellent antioxidant properties. To understand the relationship of antioxidant extractability with respect to solvents of increasing polarity, all ginger varieties were subjected to solvent extraction.

Fig. 3.1 (a – d) shows the antioxidant potential of hexane, ethanol, ethyl acetate and water extracts of all the varieties. Results of the study revealed that ethanol was the best solvent followed by ethyl acetate whose extractives gave maximum total phenolic content (TPC), total flavonoid content (TFC) as well as radical scavenging activity (RSA) for both DPPH and ABTS radicals. This could be due to the fact that most of the antioxidants present in the ginger are of phenolic nature, which has a greater affinity to polar molecules (Yeh et al., 2014). The ethanolic extracts of *Sungro-sung* variety were found to exhibit highest TPC and TFC of 0.96 and 1.52 mg g^{-1} dry weight, respectively. This was higher than the reported values of 0.76 mg total phenols g^{-1} dry weight and 1.1 mg flavonoids g^{-1} dry weight (Eleazu et al., 2013). A similar trend was exhibited in the antioxidant activities (in terms of DPPH radical) for ethanolic extracts of all the varieties. It was found to be in the range of 74.3 to 91.3 % for *Sungro-sung* variety having the highest activity followed by *Haem* variety (88.8 %, sample code 7). Eleazu et al. (2013)

TH-2388_136107006

reported 75 % inhibition of DPPH radical for their best variety which was found to be substantially lower than *Sungro-sung* variety used in this study. All DPPH values were also found to be significantly higher than that 32% of positive control. The ABTS radical scavenging activities (in terms of percentage) followed a slightly different trend. Ethanolic extract of *Thingpui* (Mizoram) variety was found to have a maximum activity of 88.3 % followed by 88 % for water extracts from *Noangphu* (Tripura) variety.

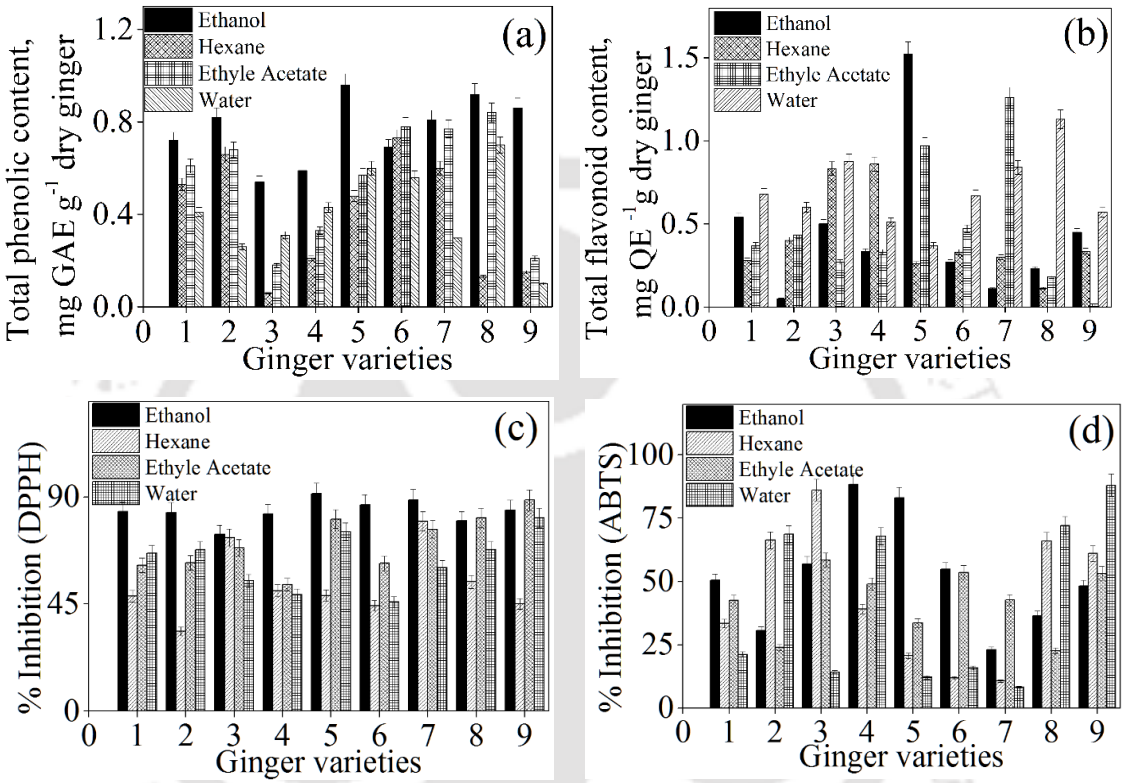


Fig. 3.1: Solvent extractives antioxidant parameters of collected ginger varieties (a) total phenolic content expressed in mg GAE g^{-1} dry ginger (b) total flavonoid contents expressed in mg QE g^{-1} dry ginger and antioxidant activities in terms of (c) % inhibition of DPPH free radical, and (d) % inhibition of ABTS free radical

However, *Sungro-sung* variety scored inhibition of 83 % which was slightly less in case of overall antioxidant activities of ABTS radical for all varieties and significantly higher than 16 % for positive control. Various researchers have demonstrated the effectiveness of ethanol over other solvents (water, hexane, acetone, ethyl acetate etc.) as the best solvent capable to extract the maximum antioxidant principles from ginger (Rahath Kubra et al., 2013; Yeh et al., 2014). In addition to this, ethanol offers a green alternative to other organic solvents for the production of lipid-based extracts. Results of this study revealed that *Sungro-sung* variety of ginger exhibits best overall antioxidant

properties compared to other varieties. This is supported by its highest total phenolic and flavonoid contents. In addition to this, the maximum fat content of *Sungro-sung* variety also supports its highest antioxidant potential since the majority of ginger's antioxidant principles are lipid in nature (Govindarajan and Connell, 1983; Kubra and Rao, 2012; Semwal et al., 2015; Srinivasan, 2017; Varakumar et al., 2017a).

3.6.2 Oleoresin and essential oil profile

Table 3.7 shows the antioxidant potential profile of all the nine varieties of dry ginger in terms of the quantity and quality of the oleoresin and essential oils extracted from them. Ginger oleoresin contains non-volatile pungent principles like gingerols, shogaols, zingerone, and paradols. Out of these, 6-gingerol is the most abundantly found ingredient. It has a wide range of pharmacological effects and hence its concentration in oleoresin is considered as a direct measure of the quality of any ginger variety traded the world over (Govindarajan and Connell, 1983; Kubra and Rao, 2012; Semwal et al., 2015).

As per the results of previous section, ethanolic extracts of ginger exhibited maximum antioxidant values hence it was used as the best solvent for oleoresin extraction. For all the varieties, the oleoresin yields and 6-gingerol contents were found to significantly vary ($p<0.5$) between 2.6 to 9.8 wt % and 0.19 to 2.2 wt %, respectively on a dry weight basis. Here again, *Sungro-sung* variety scored the highest values of oleoresin yield and 6-gingerol contents, which were found to be 1.4 and 2.8 times higher than the reported values of 7 wt % and 0.78 wt %, respectively (Bartley and Foley, 1994; Ghasemzadeh et al., 2015; Govindarajan and Connell, 1983; Kiran et al., 2013; Varakumar et al., 2017b). Thus, *Sungro-sung* variety used in this study can be considered as the richest source of 6-gingerol among the reported ginger varieties of the world. The results are also supported by the highest antioxidant activities, total phenolic and flavonoid contents of extractives, as well as the highest fat contents among all the nine varieties considered in this study (**Fig. 3.1** and **Table 3.2**).

The volatile oil yield of all the samples was found in the range of 0.43 to 0.85 wt % (dry weight basis). The major volatile actives like zingiberene, β-phellandrene, β-sesquiphellandrene, camphene and β-bisabolene for all the varieties were found to be in the range of 17.9 to 30.3 wt %, 0.34 to 2.7 wt %, 7.7 to 17.9 wt %, 0.8 to 2.4 wt % and 3.3 to 8.9 wt % of volatile oil, respectively. *Sungro-sung* variety was found to have the maximum volatile oil content along with zingiberene and β-bisabolene.

TH-2388_136107006

TH-2386_136107006

Table 3.7: Oleoresin and volatile oil profiles of all ginger varieties

Varieties	Oleoresin		Volatile oil					
	Yield, %	6-gingerol content, %	Yield, %	zingiberene, %	β-phellandrene, %	β-sesquiphellandrene, %	camphene, %	β-bisabolene, %
1	9.6 ± 0.3^{f}	0.61 ± 0.2^{d}	0.61 ± 0.2^{c}	20.3 ± 0.7^{c}	0.71 ± 0.2^{b}	10.6 ± 0.3^{c}	1.96 ± 0.06^{d}	1.84 ± 0.06^{a}
2	5.4 ± 0.3^{d}	0.82 ± 0.3^{e}	0.62 ± 0.2^{c}	18.7 ± 0.6^{ab}	1.21 ± 0.3^{e}	7.7 ± 0.3^{a}	1.68 ± 0.03^{c}	4.73 ± 0.15^{d}
3	7.6 ± 0.2^{e}	1.7 ± 0.5^{g}	0.8 ± 0.2^{g}	24.8 ± 0.8^{e}	2.66 ± 0.8^{i}	17.9 ± 0.6	2.4 ± 0.08^{f}	5.87 ± 0.19^{f}
4	3.3 ± 0.1^{b}	0.21 ± 0.1^{a}	0.74 ± 0.3^{f}	23.4 ± 0.7^{d}	1.05 ± 0.3^{d}	13.5 ± 0.4^{f}	0.79 ± 0.02^{a}	3.25 ± 0.11^{b}
5	9.8 ± 0.4^{f}	2.21 ± 0.6^{h}	0.85 ± 0.3^{h}	30.3 ± 1.2^{f}	1.87 ± 0.6^{h}	15.7 ± 0.5^{f}	2.22 ± 0.07^{e}	7.97 ± 0.25^{g}
6	4.5 ± 0.8^{c}	0.19 ± 0.1^{a}	0.65 ± 0.2^{d}	19.5 ± 1^{bc}	0.97 ± 0.3^{c}	12.9 ± 0.5^{f}	0.88 ± 0.03^{b}	4.27 ± 0.14^{c}
7	2.6 ± 0.1^{a}	0.28 ± 0.1^{b}	0.71 ± 0.1^{e}	25.8 ± 0.9^{e}	1.41 ± 0.3^{f}	10.6 ± 0.4^{c}	1.96 ± 0.06^{d}	8.92 ± 0.29^{h}
8	8.2 ± 0.2^{e}	0.33 ± 0.1^{c}	0.56 ± 0.2^{b}	22.3 ± 0.8^{d}	0.34 ± 0.1^{a}	11.8 ± 0.2^{d}	2.16 ± 0.07^{e}	5.28 ± 0.16e
9	4.9 ± 0.3^{cd}	1.03 ± 0.3^{f}	0.43 ± 0.1^{a}	17.9 ± 0.8^{a}	1.79 ± 0.5^{g}	9.6 ± 0.4^{b}	0.86 ± 0.02^{ab}	3.44 ± 0.11^{b}

subscript letters within the same column represent significant differences at $p<0.05$

This was followed by *Arunachal Pradesh local* variety which had 0.8 % volatile oil content and highest β-phellandrene, β-sesquiphellandrene and camphene contents. The volatile oil content in our study was found to be lower than the reported values of 1 – 2.5 wt % for the major commercial varieties of the world (Govindarajan and Connell, 1983; Parthasarathy et al., 2008). However, the zingiberene, β-sesquiphellandrene, and β-bisabolene contents in the current study were found to be higher than the reported contents of 29.9 wt %, 10.1 wt %, and 6.2 wt % of volatile oil, respectively, in a study of essential oil quality of ten northeast Indian ginger varieties (Kiran et al., 2013). Interestingly, the zingiberene content of *Sungro-sung* variety (30.3 wt %) was found to be slightly higher than the reported value of 29.5 wt % of Nigerian variety which is considered among the best varieties of the world (Govindarajan and Connell, 1983; Parthasarathy et al., 2008).

Table 3.8 presents the physical properties of volatile and non – volatile (oleoresin) oils of all the varieties. The refractive index and specific gravity values for both the oils were found to be in the range of 1.46 to 1.49 and 0.86 to 0.89, respectively which are consistent with the reported values of 1.47 (Parthasarathy et al., 2008). The optical rotation varied from -28.1 ° to -31.7 ° and -32.5 ° to -40.5 ° for volatile and non – volatile oils, respectively which showed that both oils were laevorotatory. This value is used as a direct measure of the freshness of the oil samples traded around the world. It is found to be abnormally low for the oils which have been distilled from old material or in samples which have been improperly stored (exposed to light, air and high temperature) and also in adulterated samples (Parthasarathy et al., 2008). The physical appearance of volatile oil was yellow for *Gangtok local*, *Imphal Local* and *Noangphu* varieties, and light yellow for *Arunachal Pradesh local*, *Thingpui*, *Sungro-sung*, *Haem* and *Malang* varieties. This conforms to international specifications (EOA-13 and Indian standard 761-1955) which are used for global trade (Parthasarathy et al., 2008). The orangish-yellow color of *Karbi Anglong* variety could be due to the presence of some unwanted artifacts. The non – volatile oil had a viscous dark brown appearance for all the varieties, similar to the reported data on oleoresin quality (Govindarajan and Connell, 1983).

Table 3.9 presents the correlations between the oleoresin and volatile oil profiles of all the nine ginger varieties of northeast India. The oleoresin yields and 6-gingerol content were positively correlated (r = 0.68).

TH-2388_137107006

TH-2386_136107006

Table 3.8: Physical properties of volatile and non-volatile oils of different varieties of ginger

Parameters	Ginger varieties								
	1	2	3	4	5	6	7	8	9
	Volatile Oil								
Refractive index	1.49 ± 0.05	1.47 ± 0.04	1.48 ± 0.05	1.49 ± 0.06	1.48 ± 0.05	1.46 ± 0.04	1.49 ± 0.05	1.47 ± 0.05	1.47 ± 0.04
Specific gravity	0.87 ± 0.03	0.89 ± 0.04	0.88 ± 0.03	0.87 ± 0.04	0.89 ± 0.04	0.88 ± 0.03	0.86 ± 0.02	0.88 ± 0.03	0.87 ± 0.04
Optical rotation, °	-30.5 ± 1.11	-31.7 ± 1.25	-28.1 ± 1.12	-30.5 ± 1.17	-29.5 ± 1.19	-30.5 ± 1.22	-30.2 ± 1.21	-30.8 ± 1.23	-30.1 ± 1.2
Appearance	Yellow	Yellow	Light Yellow	Light Yellow	Light Yellow	Orangish Yellow	Light Yellow	Light Yellow	Yellow
	Non – Volatile Oil								
Refractive index	1.49 ± 0.06	1.49 ± 0.05	1.48 ± 0.04	1.47 ± 0.05	1.48 ± 0.04	1.49 ± 0.07	1.49 ± 0.07	1.49 ± 0.06	1.49 ± 0.07
Specific gravity	0.86 ± 0.01	0.87 ± 0.03	0.89 ± 0.04	0.87 ± 0.03	0.87 ± 0.04	0.87 ± 0.04	0.86 ± 0.02	0.87 ± 0.03	0.87 ± 0.02
Optical rotation, °	-35.5 ± 1.31	-38.2 ± 1.53	-37.1 ± 1.45	-33.5 ± 1.34	-32.5 ± 1.28	-35.7 ± 1.41	-32.5 ± 1.3	-40.5 ± 1.64	-38.6 ± 1.58
Appearance	viscous dark brown								

TH-2388_07707006

Table 3.9: Pearson correlation coefficients (r) between oleoresin and volatile oil parameters of different ginger varieties

Parameter	Oleoresin yield	6-gingerol	Essential oil yield	Zingiberene	β-phellandrene	β-sesquiphellandrene	Camphene
6-gingerol	0.68*						
Essential oil yield	0.30	0.47					
Zingiberene	0.33	0.52	0.84*				
β-phellandrene	0.16	0.75*	0.41	0.36			
β-sesquiphellandrene	0.17	0.25	0.67*	0.62	0.25		
Camphene	0.69*	0.49	0.48	0.56	0.22	-0.02	
β-besabolene	0.01	0.34	0.56	0.74*	0.38	0.11	0.52*

*significant at $p < 0.05$

Guwahati

This is because 6-gingerol is a lipid-soluble compound and hence is an important quality determinant for oleoresins extracted from ginger (Govindarajan and Connell, 1983; Ismail and Lingamallu, 2012; Semwal et al., 2015). Surprisingly, the correlations of oleoresin and camphene contents (r = 0.69) and 6-gingerol with β-phellandrene contents (r = 0.75) was unique for all the varieties. The essential oil yields were found to strongly correlate with zingiberene (r = 0.84) and β-sesquiphellandrene (r = 0.67) contents. This could be because both of these compounds are major volatile actives and have identical antioxidant and antimicrobial effects. Because of this reason, their content in volatile oil samples are used as a quality marker globally (Govindarajan and Connell, 1983; Parthasarathy et al., 2008). A similar relationship was found within other major volatile actives for all the varieties like β-bisabolene and zingiberene (r = 0.74) and with camphene contents (r = 0.52).

3.7 Principal component analysis (PCA)

Multivariate data analysis tool, PCA, was used to examine the nutritional and antioxidant data of all the ginger varieties to visualize the response patterns in the feature space of principal components (PCs). To address the complexities associated with large data of the present systems, PCA was used to reduce the multi-dimensional data sets. It generates an orthogonal transformation of the raw data in the form of PCs, which is easier to interpret than the original data. The prioritization of the principal components is based on the coverage of most of the explained variance within the variables. **Table 3.10** shows the extracted PCs, their eigen values and covered percentage of variance for both data types. **Fig. 3.2** represents the principal component biplot which emphasized the difference between the measured responses (nutritional and antioxidant attributes) by separately grouping the ginger varieties. In **Fig. 3.2(a)**, PC1 and PC2 accounted for 50.94 and 17.76 % of the variation in the nutritional parameters. Similarly, in **Fig. 3.2(b)**, PC1 and PC2 described 44.35 and 22.39 % of antioxidant parameters for all the nine varieties. Cumulatively they represented 68.7 % of the entire nutritional and 66.74 % of antioxidant parameter dataset respectively, covering the maximum variation of all the parameters. **Table 3.11** shows that for nutritional parameters, the PC1 was mainly governed by the starch, total sugars and reducing sugars contents.

As a result, these parameters were found clustered together near the x-axis of the first quadrant of Fig. 3.2(a) along with the varieties having similar values of PC score

Table 3.10: Eigen values of extracted principal components of nutrition and antioxidant parameters for different varieties of ginger

Principal component	Eigen value	Percentage of variance	Cumulative %
Nutrition parameters			
1	4.08	50.94	50.94
2	1.42	17.76	68.70
3	1.08	13.46	82.16
4	1	12.53	94.69
5	0.24	2.94	97.64
Antioxidant parameters			
1	3.55	44.35	44.35
2	1.79	22.39	66.74
3	1.19	14.91	81.65
4	1.02	12.74	94.39
5	0.28	3.48	97.87

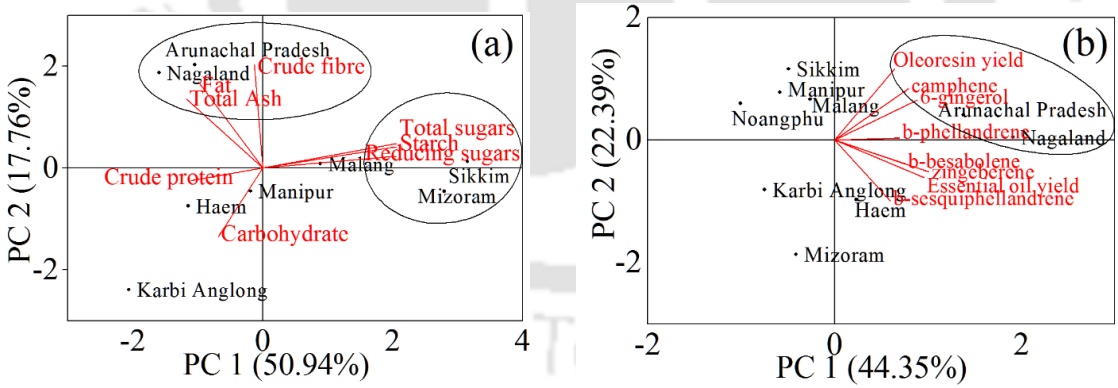


Fig. 3.2: Principal components biplot of different ginger varieties and their (a) nutrition parameter scores (b) antioxidant parameter scores

data (*Gangtok local* and *Thingpui*). Similarly, PC2 represented crude fiber, fat and total ash contents, therefore these properties along with the varieties having similar contents of these parameters (*Arunachal Pradesh local* and *Sungro-sung*) were found near y-axis of the second quadrant. Similarly, for antioxidant parameters, as presented in **Fig. 3.2(b)**,

TH-2388_137707006

PC1 best represented the essential oil yield and zingiberene contents (**Table 3.11**), whereas oleoresin yield, 6-gingerol, and camphene contents were best denoted by PC2. Hence, these were found clustered in the biplot space of x and y-axis respectively.

Table 3.11: Coefficients of extracted principal components of nutrition parameters for different varieties of ginger

Parameters	Coefficients of PC1	Coefficients of PC2
Nutritional parameters		
Crude fibre	-0.03	0.61
Crude protein	-0.27	-0.07
Starch	0.5	0.13
Total sugars	0.5	0.14
Reducing sugars	0.5	0.07
Total Ash	-0.29	0.4
Carbohydrate	-0.17	-0.4
Fat	-0.24	0.5
Antioxidant parameters		
Oleoresin yield	0.28	0.56
6-gingerol	0.39	0.31
Essential oil yield	0.42	-0.3
Zingiberene	0.44	-0.25
β-phellandrene	0.31	0.02
β-sesquiphellandrene	0.26	-0.48
Camphene	0.35	0.4
β-bisabolene	0.33	-0.19

Two distinct groups were formed, *Arunachal Pradesh local* and *Sungro-sung* varieties were grouped together towards x-axis whereas, *Gangtok Local, Noangphu, Malang* and *Imphal Local* were found clustered near y-axis. Other properties and varieties had less correlated properties and were hence found scattered throughout the biplot space. Hence from the PC biplots, it can be clearly concluded that *Sungro-sung*

and *Arunachal Pradesh local* varieties had similar nutritional and antioxidant properties. This is also supported by the results of solvent extractives, **Fig. 3.1** along with the oleoresin and volatile oil profiles, **Table 3.7**.

3.8 Hierarchical cluster analysis (HCA)

As detailed in the previous section, PCA evidently segregated the ginger varieties data according to their nutritional and antioxidant compositions, but could not illustrate the groupings of ginger varieties prominently. That was because of the substantial spread of variation along PCs due to the varying components of dissimilar nutritional and antioxidant nature. Hence, cluster analysis was performed followed by PCA, to group the data according to their similarity, forming three and two distinct clusters for nutritional and antioxidant parameters, respectively, each one representing regions of the identical properties.

HCA is a powerful chemometric tool used to discover the inherent grouping and distribution of the underlying data (Upadhyay and Mishra, 2015; Upadhyay et al., 2017). **Fig. 3.3** presents the dendrograms for all the parameters of the nine ginger varieties obtained by applying the city block distance and furthest neighbor method. **Fig 3.3(a)** shows the dendrogram of ginger varieties for their nutritional composition. The varieties have been grouped into three well-defined clusters as I (*Gangtok local*, *Noangphu* and *Thingpui* varieties), II (*Arunachal Pradesh local* and *Sungro-sung* varieties), and III (*Imphal Local*, *Karbi Anglong*, *Haem* and *Malang varieties*). These groups have been found to have ginger varieties containing similar nutritional parameters. Group II is found to have both the varieties lying at same cluster distance signifying that *Sungro-sung* and *Arunachal Pradesh local* varieties have most similar nutritional properties (supported by nutritional data of **Table 3.2**). This is also supported by the PC biplot, **Fig 3.2(a)** which shows similar PC scores for both these varieties and, as a result, they were found clustered together. Similarly, **Fig 3.3(b)** shows the dendrogram of ginger varieties for their antioxidant compositions. This consists of two major clusters with the group I containing *Gangtok local*, *Malang*, *Imphal Local*, *Noangphu*, *Karbi Anglong*, *Thingpui* and *Haem* varieties. Group II was unique with only two varieties *Arunachal Pradesh local* and *Sungro-sung*, having most similar antioxidant profiles. The results are in accordance with **Fig. 3.2(b)** which clearly group these two varieties for their similar values of oleoresin yield, 6-gingerol, and camphene content. In addition to the PC plots, the results of antioxidant profiles also clearly show that *Sungro-sung* and *Arunachal*

TH-2388_137107006

Pradesh local varieties have higher values, with former variety having maximum antioxidant potential.

Thus, the chemometric tools (PCA and HCA) clearly segregated the ginger varieties selected for study in different groups with similar content of nutritional and antioxidant properties. Thus, this study can act as a basis for deciding the best candidate for a range of industrial applications specific for dry and fresh rhizomes.

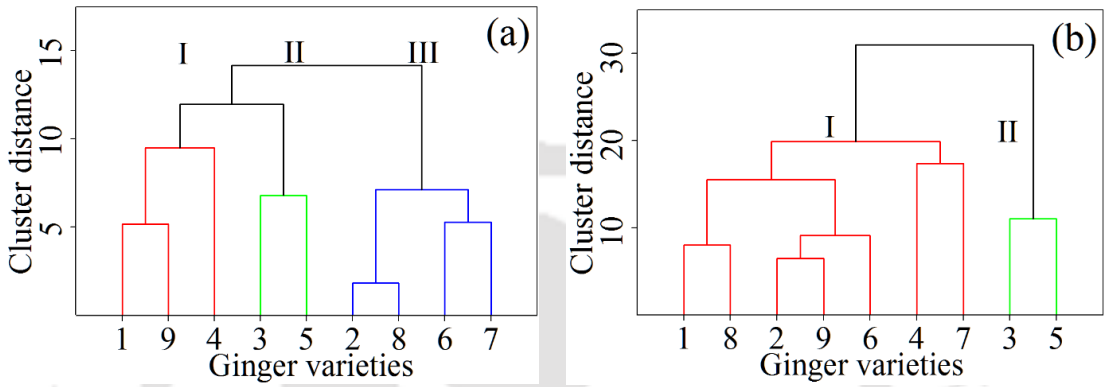


Fig. 3.3: Dendrogram of hierarchical cluster analysis for (a) nutritional parameters (b) antioxidant parameters of all ginger varieties

3.9 Conceptual mapping among physical, nutritional and antioxidant parameters

Among all the parameters, the commercial value of ginger is often evaluated majorly in terms of its antioxidant parameters namely 6-gingerol and volatile oil contents. Pearson's correlation analysis was performed for all the estimated physical, nutritional and antioxidant parameters pertaining to all the nine varieties.

Table 3.12 summarizes important correlations among all the properties. It can be clearly seen that fat content is strongly correlated with 6-gingerol (a major active ingredient of oleoresin) as well as zingiberene and β-bisabolene (a major ingredient of volatile oil) for all the ginger varieties. Average size and weights are also found to be inversely correlated with fat, 6-gingerol, zingiberene and β-bisabolene contents of all the varieties.

Thus, it can be concluded that fresh ginger rhizomes of smaller size had higher fat, 6-gingerol, zingiberene and β-bisabolene contents and hence, in general, overall higher antioxidant properties. Few researchers have attempted to give an easy and time saving non-destructive methods for example in case of quality evaluation of fresh apples (Jha et al., 2012). However, such tools have never been reported for ginger varieties.

This observation can potentially act as a simple screening criterion for instantaneously deciding the antioxidant quality of ginger for industrial applications. Thus, a lot of time could be saved in the complete process of firstly drying the rhizomes followed by extracting oleoresin and volatile oil and thereafter quantifying them with expensive and sophisticated techniques like HPLC and GCMS.

Table 3.12: Conceptual mapping among physical, nutritional and antioxidant parameters

Correlated parameters	Extent of correlation
Direct correlation	
Fat – 6-gingerol	+++
Fat – Zingiberene	+++
Fat – β-bisabolene	+++
Crude Fibre – 6-gingerol	+++
Total Flavonoids – 6-gingerol	+++
Total Ash – 6-gingerol	++
6-gingerol – Copper	++
6-gingerol – Nickel	++
Zingiberene – Nickel	++
Inverse correlation	
Average Size – Fat	+++
Average Weight – Fat	+++
Average Size – 6-gingerol	+++
Average Weight – 6-gingerol	+++
Average Size – Zingiberene	+++
Average Weight – β-bisabolene	+++
Water Retention Capacity – Camphene	+++
Swelling Capacity – Magnesium	+++

TH-2388_137107006

3.10 Literature comparison

Table 3.13 presents a comparison of the present study results with published literature covering major ginger varieties of the world. 6-gingerol is the most abundant active ingredient found in ginger, hence it is widely accepted as a single quality determining parameter. It can be clearly seen that *Sungro-sung* variety from Nagaland has 2.2 wt % of the 6-gingerol content (dry weight basis) which is higher than all the reported values. *Arunachal Pradesh Local* of the current study also had a higher content than most of the reported values except for *Chu-ginger* from Taiwan which had 1.9 wt % (Yeh et al., 2014). Apart from the antioxidant parameters, very few studies are available dealing exclusively with the nutritional parameters of ginger. It can be seen from the **Table 3.13** that *Sungro-sung* variety has 18 wt % fiber which is higher than the reported values of 9.9 wt % of *Supraya* variety. However, the protein content in the current study was less than the reported value of 19 wt % of *Kuruppampadi* variety (Pattnaik et al., 2016).The Mn content (129.1 ppm) of *Sungro-sung* was also found to be higher as compared to 118 ppm of a variety (sample code 113) from north India (Pandotra et al., 2015). As detailed in the above sections, the data on other properties of ginger remains largely unexplored and hence could not be compared. In addition to this, most of the published reports on ginger characterization have not systematically studied the complete spectrum of data (physical, macro and micronutrients, antioxidant properties and bioactive compounds) or have not screened the industrially important varieties using tools like PCA and HCA (Adel and Prakash, 2010; Kiran et al., 2013; Pawar et al., 2011; Sanwal et al., 2010; Yudthavorasit et al., 2014).

3.11 Summary

The chapter presents comprehensive database of a range of chemical and physical properties of nine different varieties of ginger collected from various parts of northeast India. The conclusions are summarized as follows:

- The relative abundance of mineral contents followed a trend of K > Na > Mg > Ca > Fe > P > Mn > Zn > Cr > Ni > Cu for all the varieties.
- Ethanol proved to be the best solvent for extracting maximum phenolic (0.96 mg g^{-1}) and flavonoids (1.52 mg g^{-1}) content from dried rhizomes.
- *Sungro-sung* variety from Nagaland was found to contain highest 6-gingerol content (2.2 wt % on dry basis).

TH-2388_08307006

Table 3.13: Comparison of ginger quality of present work with various published reports

Varieties considered	Origin	Screening tools used	Parameters studied	Best Variety	Best Parameters (on dry wt. basis)	References
Antioxidant properties based studies						
9	Northeast India	PCA, HCA and correlation based screening of best varieties for industrially relevant physically, nutritional and antioxidant parameters	6 physical, 8 macronutrient, 11 micronutrient and 16 antioxidant parameters	*Sungro-sung* and *Arunachal Pradesh Local*	2.2% 6-gingerol	**Current study**
10	Northeast India	HCA based clustering of varieties having similar antioxidant parameters	3 antioxidant parameters	Tripura-II and Nagaland Nadia	1.7 % 6-gingerol	(Kiran et al., 2013)
12	Pan India	HCA based clustering of varieties having similar antioxidant parameters	4 antioxidant parameters	Rio and Rajasthan	0.2 % 6-gingerol	(Pawar et al., 2011)
4	Jamaica	None	2 antioxidant parameters	Bulbous Blue Ginger	1.6 % 6-gingerol	(Salmon et al., 2012)
2	Taiwan	None	4 antioxidant parameters	Chu-ginger	1.9 % 6-gingerol	(Yeh et al., 2014)
Nutritional properties based studies						
		Current Study		*Sungro-sung*	18 % fiber, 17 % protein, 2.7% fat, 129.1 ppm Mn	Current Study
20	East and South India	PCA and HCA for grouping similar varieties	Biochemical properties - protein, phenol polyphenol oxidase and fiber content	Suprava and Kuruppampadi	9.9 % fiber, 19 % protein	(Pattnaik et al., 2016)
46	Northern India	PCA and HCA for grouping similar varieties	16 Mineral contents	110 and 113	52.5 ppm Zn, 118 ppm Mn	(Pandotra et al., 2015)

- Chemometric tools clearly segregated *Sungro-sung* followed by *Arunachal Pradesh local* varieties as the best in terms of overall antioxidant as well as nutritive potential.
- The correlation study within all the properties revealed a strong inverse relationship among the average size with major bioactive constituents of oleoresin and volatile oils of all the varieties. This single parameter can potentially be used as a rapid inexpensive non-destructive method in developing countries for screening the ginger varieties for commercial production of volatile oil and oleoresin.

भारतीय प्रौद्योगिकी संस्थान गुवाहाटी • Indian Institute of Technology Guwahati •

TH-2386_136107006

Chapter 4

Parametric optimality of supercritical fluid extraction-cum-fractionation technology for isolation of gingerols enriched oleoresin and high quality essential oil from best ginger variety

Work published at:

Shukla, A., Naik, S. N., Goud, V. V., & Das, C. (2019). Supercritical CO_2 extraction and online fractionation of dry ginger for production of high-quality volatile oil and gingerols enriched oleoresin. *Industrial Crops and Products*, *130*, 352-362.

TH-2386_136107006

TH-2386_136107006

4.1 Background

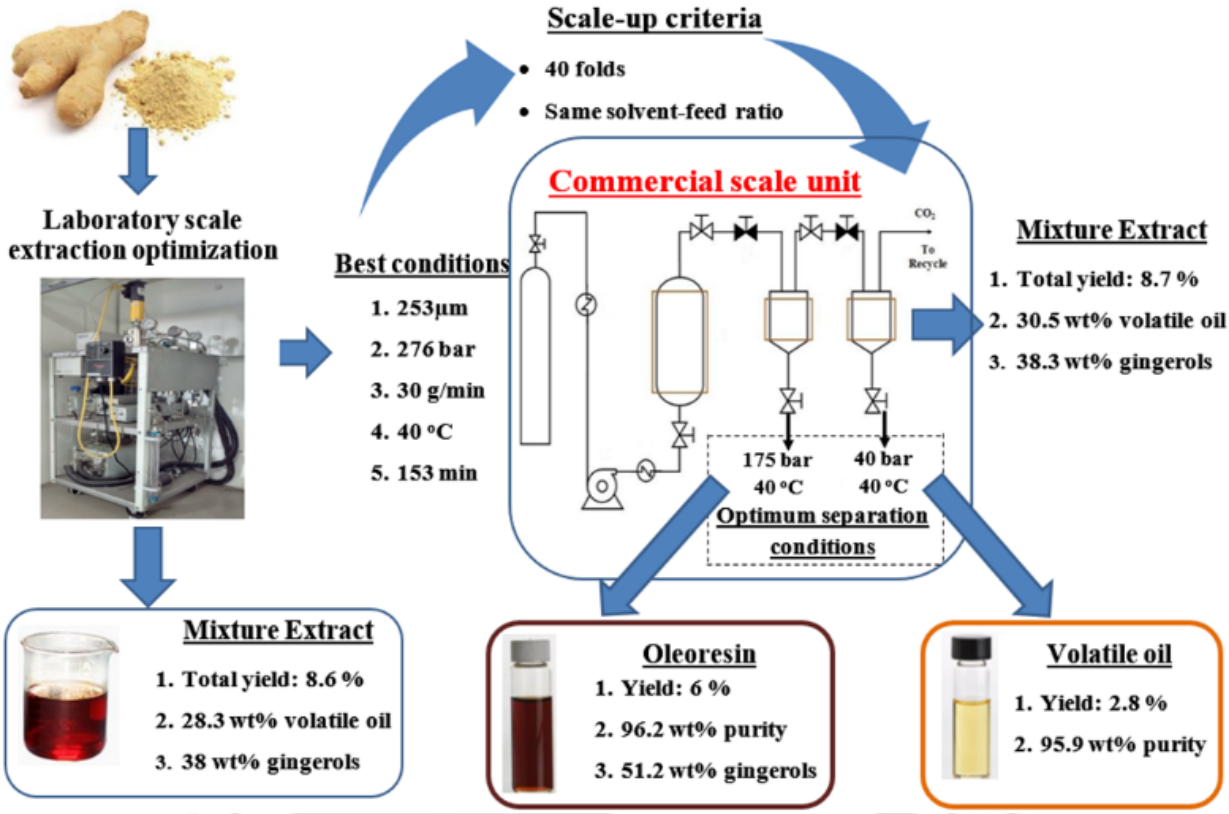
Conventional extraction techniques are still the most common methods for commercial production of ginger extracts from dry ginger. These are however associated with major drawbacks such as complex processes involving multiple unit operations (extraction, separation etc.), high operating temperatures which cause degradative changes to major active compounds and their yields. Apart from this, the use of organic solvents has been reported to be hazardous on health while some solvents have shown carcinogenic effects. Supercritical CO_2 ($SCCO_2$) is a promising solvent owing to its low critical pressure of 73.8 bar and temperature of 31 ^oC. Heat sensitive compounds can be extracted by it without any degradation and it is an environmentally acceptable solvent which does not introduce any trace of harmful chemicals. Hence in this respect, it is attempted to develop a green process for isolating ginger extracts, namely, ginger oleoresin and volatile oil of highest possible quality from dry ginger using $SCCO_2$.

4.2 Overview

Ginger oleoresin and volatile oil find a wide range of applications in food, beverage, pharmaceutical, biotechnology and fragrance industry. The extraction and processing methodologies chosen are critical for overall quality of the extract as well as for quantitative measure of temperature sensitive major actives present in the extract. In this context, a single step process for supercritical CO_2 ($SCCO_2$) extraction coupled with fractionation of dry ginger for simultaneous production of gingerols rich oleoresin and volatile oil has been attempted in this chapter. The optimization and scale-up validation of this process have been achieved in three stages. Firstly, $SCCO_2$ extraction conditions were optimized for the highest yield of mixture extract containing a maximum amount of volatile oil components and major actives (sum of (6,8,10) gingerols and 6-shogaol). The optimum process variables under laboratory scale conditions were: average ginger particle size 253 µm, extraction temperature 40 $^{\circ}C$, pressure 276 bar, flow rate 30 g min^{-1} and 153 min of extraction time. This resulted in 8.6 % yield of a mixture extract having 38 wt % of major actives and 28.3 wt % of volatile oil. In the second stage of experiments, these conditions were scaled up to 50 folds on a commercial scale unit. The recovered mixture extract recorded an improvement in 0.1 % yield with an increase of 0.3 and 1.2 wt % in its major actives and volatile oil, respectively. Finally, the optimum conditions

TH-2386_136107006

of online $SCCO_2$ fractionation (coupled with same optimum extraction conditions) resulted in 5.95% oleoresin yield, which was 96.2 % pure and 51.2 wt % of major actives in separator 1 operating at 175 bar/40 ˚C. Simultaneously, 2.8 % yield of volatile oil (95.9 % pure) was recovered in separator 2 (40 bar/40 ˚C). The yield and quality of oleoresin and volatile oil extracted from conventional methods were also compared.



Graphical abstract of the work presented in this chapter

TH-2388_138407006

4.3 Results and discussion

4.3.1 Laboratory scale optimization of $SCCO_2$ extraction

4.3.1.1 Preliminary experiments for finding optimum particle size and temperature

In order to get ginger powder with optimum particle size which could result in best quality and highest yield extract, preliminary extraction experiments were carried out with ginger powder of different particle sizes. Results revealed that coarse powder (having a particle size range between 1000 to 355 µm) resulted in the lowest yield of 6.8 %, whereas fine powder with a range of 355 to 150 µm (average particle size 253 µm) significantly ($p < 0.05$) increased the yield of extract to 7.5 %. The increased surface area of smaller particles facilitated higher mass transfer rates between ginger particle matrix and $SCCO_2$ solvent. Powder with a finer range of particles (< 150 µm) resulted in increased packing density, thus reducing the solvent diffusivity and hence extract yield to 7.1 % (Said et al., 2015). Hence, ginger powder passing through a sieve having a mesh size of 355 µm was considered optimum for further studies. The result was consistent with the findings of Said et al. (2015) and Badalyan et al. (1998), who reported ginger powder with average particle size of 250 µm was optimum for highest recovery of ginger oleoresin by $SCCO_2$ extraction.

It can be seen from **Fig. 4.1(a)** that increasing temperature slightly increased the yield of oleoresin from 5.5 % at 40 ˚C to 5.8 % at 50 ˚C (statistically significant at $p<0.05$), however the change in volatile oil yield in all the studied temperatures, was non-significant. From **Fig. 4.1(b)**, it is observed that the content of gingerols in the oleoresin increased slightly from 33.8 to 34.5 wt % with temperature from 35 ˚C to 40 ˚C and thereafter decreased significantly to 31.7 wt % with a further increase in temperature to 50 ˚C. On the contrary, the change in the 6-shogaol content of recovered oleoresin was not significant for 35 and 40 ˚C. However, it significantly increased to 3.1 and 4.2 wt % at 45 ˚C and 50 ˚C, respectively, as presented in **Fig. 4.1(c)**. The temperature increase beyond 40 ˚C caused the 6-gingerol to degrade into 6-shogaol because of the dehydration at its β-hydroxy ketone group under high temperatures, which has been very well documented by George et al., (2017) and Nair, (2013). Because of this reason, the quality of oleoresin traded globally is dependent on its quantitative contents of major gingerols (6, 8, and 10) and 6-shogaol. Hence for the best quality extract, it was decided to keep the 6-shogaol content in the extract as minimum as possible. Therefore, 40 ˚C was considered the optimum temperature for all the following experiments.

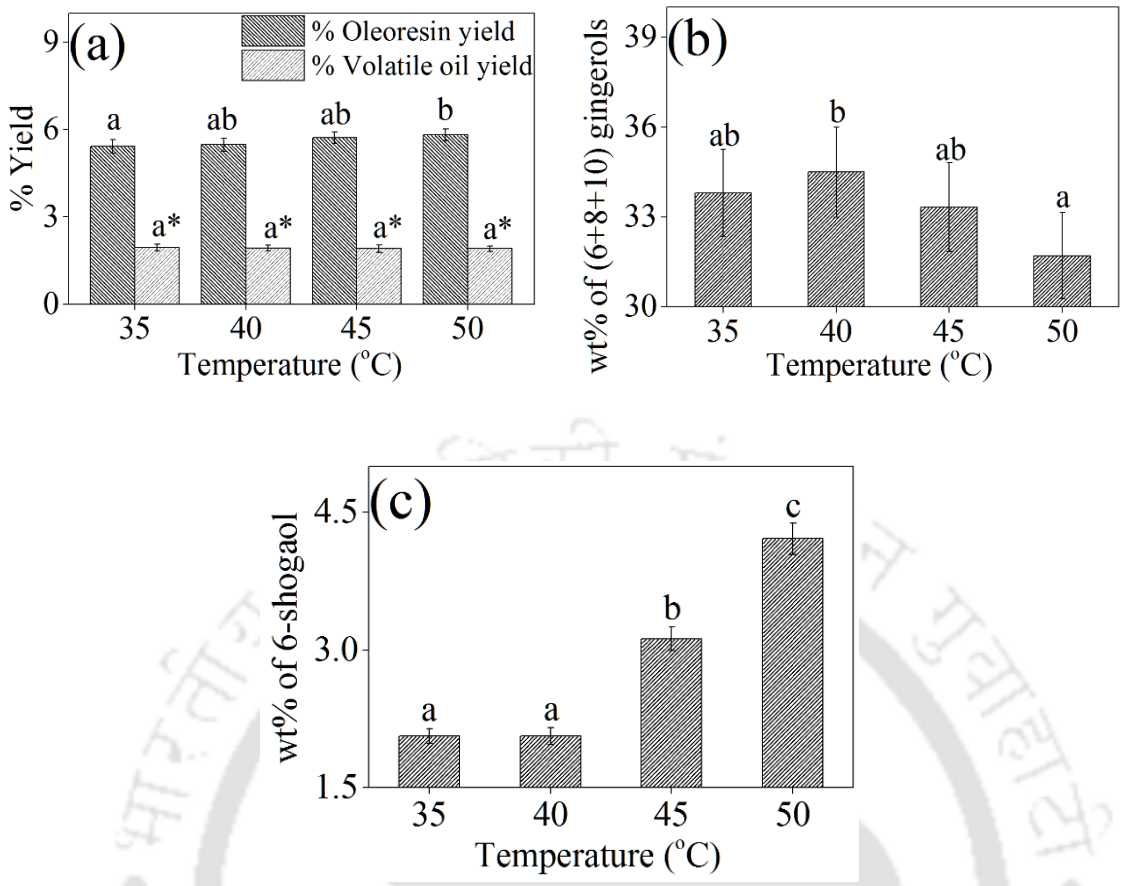


Fig. 4.1: Results of preliminary experiments (a) effect of temperature on yield of volatile oil and oleoresin (b) effect of temperature on wt % of gingerols (c) effect of temperature on wt % of 6-shogaol. Different letters indicate significant difference at $p<0.05$ (Duncan's test). Letters with asterisk (*) denote statistical significance for % volatile oil yield

4.3.1.2 Optimization of pressure, flow rate and time of extraction

Following the results of preliminary trials, the optimum values of particle size and process temperature were used for optimizing remaining process parameters in the experimental design as described in materials and methods section. The responses have been reported for each experimental point of the design matrix in **Table 4.1**. The average yield of $SCCO_2$ extract from dry ginger was within a range of 6 – 9 %. The major actives and volatile oil content of the extract varied from 23.4–37.6 wt % and 25.4–31.6 wt %, respectively.

TH-2388_13307006

Table 4.1: Experimental design matrix and actual data of performed experiments

Run	Uncoded and coded (between parenthesis) values of independent variables*			Responses		
	Pressure (bar) (A)	Flow rate (g min^{-1}) (B)	Time (min) (C)	%Yield	wt % major actives[1]	wt % volatile oil[2]
Factorial Points						
1	300.00(+1)	40.00 (+1)	180.00(+1)	8.2 ± 0.3^{de}	36.3 ± 1.8^{fgh}	26.4 ± 1^{ab}
2	300.00 (+1)	20.00 (-1)	180.00 (+1)	8.9 ± 0.2^{f}	34.6 ± 1.1^{ef}	27.1 ± 1.1^{ab}
3	150.00 (-1)	20.00 (-1)	60.00 (-1)	6 ± 0.2^{a}	26.9 ± 0.8^{b}	26.4 ± 0.9^{ab}
4	300.00 (+1)	20.00 (-1)	60.00 (-1)	7.6 ± 0.3^{bc}	31.9 ± 1.5^{cd}	27.3 ± 0.8^{ab}
5	150.00 (-1)	20.00 (-1)	180.00 (+1)	8.5 ± 0.3^{ef}	30.1 ± 1^{c}	29.6 ± 0.8^{d}
6	150.00 (-1)	40.00 (+1)	60.00 (-1)	7.3 ± 0.2^{b}	26.9 ± 1.3^{b}	26.1 ± 0.9^{ab}
7	300.00 (+1)	40.00 (+1)	60.00 (-1)	7.9 ± 0.3^{cd}	34.8 ± 1.8^{efg}	26.5 ± 1.1^{ab}
8	150.00 (-1)	40.00 (+1)	180.00 (+1)	8.1 ± 0.3^{cde}	32.9 ± 1.2^{de}	30.2 ± 0.9^{de}
Axial Points						
9	225.00(0)	13.18 (-α)	120.00 (0)	7.9 ± 0.3^{cd}	30.1 ± 0.7^{c}	27.1 ± 0.7^{ab}
10	225.00 (0)	30.00 (0)	220.91 (+α)	8.5 ± 0.3^{def}	37.6 ± 1^{h}	29.9 ± 1.1^{de}
11	225.00 (0)	30.00 (0)	19.09 (-α)	6 ± 0.2^{a}	32.9 ± 1.1^{de}	25.4 ± 0.9^{a}
12	351.13 (+α)	30.00 (0)	120.00 (0)	8.6 ± 0.3^{ef}	33.5 ± 1.1^{de}	27.8 ± 1.3^{bc}
13	225.00 (0)	46.82 (+α)	120.00 (0)	8.7 ± 0.4^{ef}	33.8 ± 1.3^{de}	27.5 ± 1.3^{bc}
14	98.87 (-α)	30.00 (0)	120.00 (0)	7 ± 0.3^{b}	23.4 ± 1^{a}	31.6 ± 0.7^{e}
Center Points						
15	225.00 (0)	30.00 (0)	120.00 (0)	9 ± 0.3^{f}	36.9 ± 1.5^{gh}	29.2 ± 0.8^{cd}
16	225.00 (0)	30.00 (0)	120.00 (0)	8.7 ± 0.3^{ef}	36.5 ± 0.9^{fgh}	30.7 ± 1^{de}
17	225.00 (0)	30.00 (0)	120.00 (0)	8.2 ± 0.4^{de}	36.4 ± 1^{fgh}	30.4 ± 0.9^{de}
18	225.00 (0)	30.00 (0)	120.00 (0)	8.3 ± 0.4^{de}	37.2 ± 1.5^{h}	29.6 ± 1.3^{d}
19	225.00(0)	30.00 (0)	120.00 (0)	8.5 ± 0.4^{ef}	36 ± 1.3^{fgh}	30.8 ± 1.5^{de}
20	225.00 (0)	30.00 (0)	120.00 (0)	8.6 ± 0.4^{ef}	36.2 ± 1.2^{fgh}	30.4 ± 1.3^{de}

1: wt % fraction of sum of (6+8+10) gingerols and 6-shogaol in extracted oleoresin; 2: wt % fraction of volatile content in oleoresin; different superscript letters within a column represent significant difference at $p<0.05$. * The range of solvent to feed ratio (S/F) was varied from 5.7 to 72.

4.3.1.2.1 Analysis of variance (ANOVA) and model fitting

For determining the suitability of models in predicting each response *i.e.* total yield (%), major actives (wt %) and volatile oil content (wt %) as a function of process parameters as well as for estimation of the coefficients of the selected models as per Eq. 4.1, regression analysis was performed. To determine the significance of each model

term, ANOVA was performed on the experimental data as shown in **Table 4.2**. The *p* values of all the selected models were found to be less than 0.0001 and for lack of fit tests were found to be 0.676, 0.126 and 0.318, for total yield (%), major actives (wt %) and volatile oil content (wt %), respectively.

Table 4.2: Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Factor	% Total yield					wt % major actives					wt % volatile Content				
	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*
Model	13.55	9	1.51	24.05	< 0.0001	293.20	9	32.58	80.98	< 0.0001	64.75	9	7.19	14.00	0.0001
A: Pressure	2.20	1	2.20	35.10	0.0001	104.12	1	104.12	258.82	< 0.0001	9.73	1	9.73	18.93	0.1014
B: Flow rate	0.25	1	0.25	3.97	0.0744	13.46	1	13.46	33.47	0.0002	0.024	1	0.024	0.048	0.8318
C: Time	6.23	1	6.23	99.54	< 0.0001	32.98	1	32.98	81.99	< 0.0001	15.62	1	15.62	30.39	0.0003
A*B	0.19	1	0.19	3.08	0.1098	0.38	1	0.38	0.95	0.3534	0.44	1	0.44	0.86	0.3757
A* C	0.33	1	0.33	5.31	0.0440	3.16	1	3.16	7.85	0.0187	7.26	1	7.26	14.12	0.0037
B* C	0.90	1	0.90	14.34	0.0036	0.36	1	0.36	0.88	0.3695	0.10	1	0.10	0.20	0.6666
A* A	0.79	1	0.79	12.58	0.0053	114.05	1	114.05	283.52	< 0.0001	1.30	1	1.30	2.53	0.1430
B* B	0.088	1	0.088	1.41	0.2627	36.05	1	36.05	89.61	< 0.0001	19.37	1	19.37	37.69	0.0001
C* C	2.93	1	2.93	46.77	< 0.0001	2.54	1	2.54	6.30	0.0309	15.14	1	15.14	29.46	0.0003
Residual	0.63	10	0.063			4.02	10	0.40			5.14	10	0.51		
Lack-of-Fit	0.25	5	0.049	0.65	0.6760	3.02	5	0.60	3.01	0.1261	3.13	5	0.63	1.56	0.3189
Pure Error	0.38	5	0.076			1.00	5	0.20			2.01	5	0.40		
R^2			0.96					0.98					0.97		
R^2 (adjusted)			0.92					0.97					0.86		
R^2 (predicted)			0.82					0.91					0.91		

To check the data fitting quality of the developed models, each experimental data point of the responses was plotted against model predicted values (**Fig. 4.2**). The R-square values for total yield (%), major actives (wt %) and volatile oil content (wt %) of the developed model were found to be 96.6, 98.7 and 97.7 %, respectively. From this statistical analysis, it was concluded that the experimental data fitted well with the developed models as given in equations (4.1) - (4.3). In addition, the quadratic model equations had a good prediction of the responses. For total yield (%), only five terms had a significant effect on the model as per the ANOVA table (**Table 4.2**). These terms were: linear terms of pressure (A) and time (C), quadratic terms of pressure and time (AC), flow rate and time (BC), pressure (AA) and time (CC).

TH-2388_13307006

$$\text{Total yield (\%)} = 8.53 + 0.4A + 0.13B + 0.68C - 0.16AB - 0.2AC - 0.33BC - 0.23A^2 - 0.078B^2 - 0.45C^2 \quad (R^2 = 0.96) \tag{4.1}$$

$$\text{Major actives (wt \%)} = 36.52 + 2.76A + 0.99B + 1.55C + 0.22AB - 0.63AC + 0.21BC - 2.81A^2 - 1.58B^2 - 0.42C^2 \quad (R^2 = 0.98) \tag{4.2}$$

$$\text{Volatile oil (wt \%)} = 30.2 - 0.84A - 0.042B + 1.07C - 0.23AB - 0.95AB + 0.11AC - 0.3A^2 - 1.16B^2 - 1.02C^2 \quad (R^2 = 0.97) \tag{4.3}$$

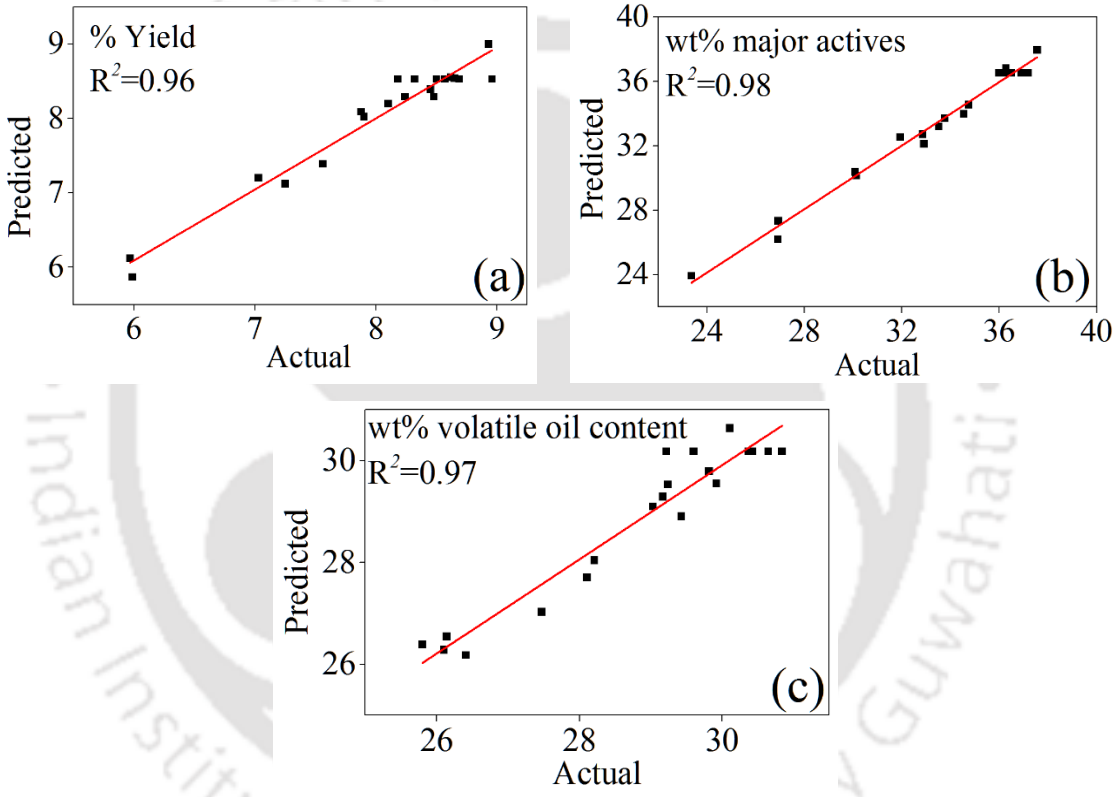


Fig. 4.2: Plots indicating deviation between actual experimental values and model predicted values of model equations for the extract (a) % yield, (b) wt % gingerols, and (c) wt % volatile content

Their *p* values were found to be 0.0001, < 0.0001, 0.044, 0.0036, 0.0053 and < 0.0001 respectively. Remaining terms did not have a significant contribution to the response ($p > 0.05$). Among the significant terms, pressure and time had similar *p*-values, however, linear term of time (C) had the largest *F*-value of 99.54 followed by a linear term of pressure (A), with *F*-value of 35.1. Hence it was concluded that the time of

extraction had the maximum effect on total yield (%) followed by pressure (Haldar et al., 2016). Similarly, pressure (A) had a significant maximum effect on the major actives content (wt %) of the extract (Eq. 4.2) and time (C) had the highest effect on volatile oil content (wt %) of the extract (Eq. 4.3).

4.3.1.2.2 Effect of process variables on response surfaces

The effect of each process variable (pressure, flow rate and time) on the responses, total yield (%), major actives (wt %) and volatile oil contents (wt %) was investigated by constructing three dimensional (3D) response surfaces of model equations (Equations 2-4). This was done by varying two process variables at a time and keeping the other constant. The total yield (%) is presented by the responses in **Fig. 4.3 (a-c)**. Time has been found to have a maximum effect on total yield (%) which was higher by 9 % towards maximum time (180 min). This could be because of the fact that in addition to major actives, higher extraction time resulted in further extraction of unwanted non-polar compounds like high chain non-cuticular waxes, resinous principles, etc. Various researchers have reported that increasing the $SCCO_2$ pressure, flow rate and contact time with ginger powder (extraction time) results in the rapid increase in diffusivity and hence increased solvating properties of $SCCO_2$ even for larger molecules from plant matrix (Reverchon, 1997; Reverchon and De Marco, 2006). This could be the reason for the higher yield of mixture extract towards the higher end of the selected range of pressure (150-300 bar), flow rate (20-40 g min^{-1}) and extraction time (60-180 min).

It is noteworthy that the mixture of extract recovered, mainly consisted of non-volatile components (76.7–62.4 wt %). These compounds were mainly comprised of major actives which have a large chain molecular structure like gingerols, shogaols, paradols, zingerones etc. (Varakumar et al., 2017). Because of their large structure, the non-volatile molecules have in general lower solubility in $SCCO_2$. Therefore, their extractability becomes more prominent at higher pressure except for small portions of cuticular waxes which are extracted at all conditions of pressure (Reverchon and De Marco, 2006). Because of these reasons, it can be seen that the response behaviour of the total yield (%) of mixture extract is quite similar to the major actives (wt %) as presented in **Fig. 4.3 (d-e)**.

TH-2388_13907006

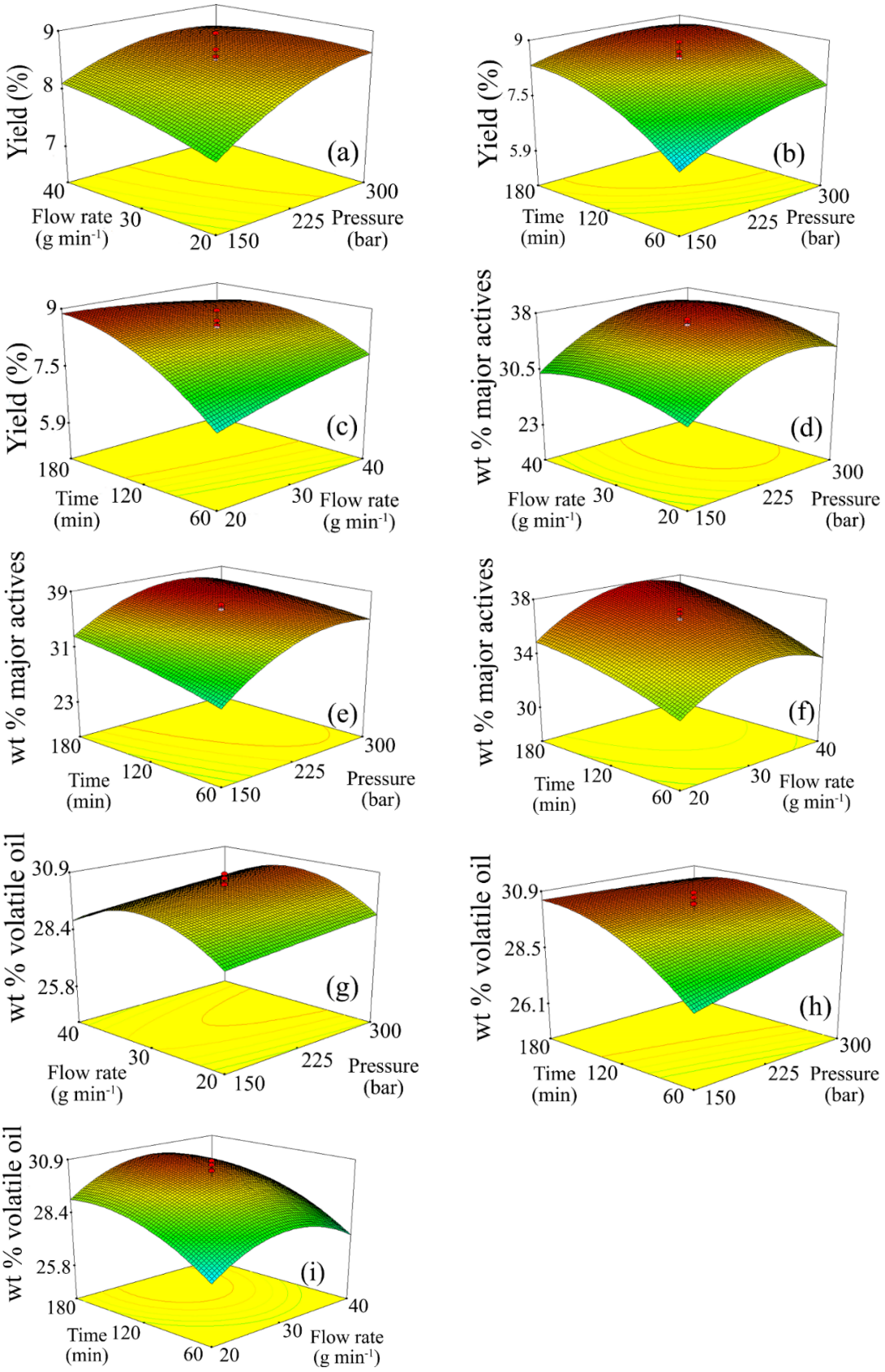


Fig. 4.3: Effect of flow rate, pressure and time on % yield recovery (a-c), on wt % major actives (d-f), and wt % volatile oil (g-i)

The pressure was found to be a most important factor in extracting major actives (sum of (6+8+10)-gingerols and 6-shogaol content) from ginger powder (**Table 4.2**) and it was found to have a maximum effect around 270 bar as presented in **Fig. 4.3 (d-e)**.

Conversely, volatile compounds are more soluble at lower ranges of $SCCO_2$ pressure because of their smaller molecular structure. Hence, the commercial extraction of volatile oil from the vegetable matrix is conducted at a lower range of pressure and flow rates (Naik et al., 1989; Yonei et al., 1995). Because of this fact, the parameters of pressure and flow rate have non-significant (p>0.05) effect on volatile oil contents (wt %) of the extract (**Table 4.2**). This fact is evident from the response surfaces in **Fig. 4.3 (g-i)**, where volatile oil content is found to decrease from 30.5 to 28 wt % with an increase in pressure from 150 to 300 bar. However, the most significant effect on response surfaces is shown by time (**Table 4.2**).

4.3.1.2.3 Numerical optimization of process parameters

Desirability function tool in Design Expert software (7.0.0) was used to predict an optimum set of independent variables (process parameters) within the chosen operating range with the goal of maximizing the dependent parameters (Haldar et al., 2016). The maximum extract yield predicted by the model was 8.9 % having 37.6 wt % of major actives and volatile oil content of 30.6 wt %. The predicted optimum process parameters for this extract were 276 bar, 30 g min^{-1} and 153 min with the desirability of 0.97. The experiments were performed at suggested optimum process conditions and the characteristics of the extract recovered were: 8.6 % yield with 38 wt % of major actives and volatile oil content 28.3 wt %. This experimental data was within a range of 4 % deviation from the predicted values which suggested the high accuracy of the model predictions.

4.3.2 Scale-up experiments

4.3.2.1 Validation of laboratory scale extraction parameters

Various researchers have reported different scale-up criteria while transferring laboratory scale optimized parameters to a large scale $SCCO_2$ facility meant for commercial production. An easy and efficient criterion used for scaling up of a $SCCO_2$ extraction process involves keeping the ratio of $SCCO_2$ solvent used for extraction to the feed (S/F ratio) constant (Paula et al., 2016; Salea et al., 2017). In this study, the optimum parameters of laboratory scale were achieved at an S/F ratio of 45.9, which was

TH-2388_13907006

maintained constantly in the commercial scale unit for obtaining the same desired quality of extract. To check the validity of laboratory scale optimized parameters in the commercial scale unit, $SCCO_2$ extraction was conducted in a large scale unit at the same optimized parameters.

Table 4.3 compares the results of laboratory scale and commercial scale experiments. An improvement of 0.1 % in total yield of mixture extract with a 0.3 wt % increase in major actives and 1.2 wt % increase in volatile oil content respectively was recorded in the mixture extract recovered in the single separator of the commercial scale unit. This slight improvement was not significantly higher from the laboratory scale results implying excellent repeatability of yield and quality characteristics in the extracts produced from both laboratory and commercial scale apparatus.

Table 4.3: Comparison between laboratory and commercial scale $SCCO_2$ extraction

Extractor volume (L)	Raw material used (g)	Process parameters					S/F	Yield (%)	Major actives (wt %)	Volatile oil (wt %)
		T (^oC)	ρ_{avg} (µm)	P (bar)	Q_{CO2} (g min^{-1})	Time (min)				
Laboratory scale										
0.5	100	40	253	276	30	153	45.9	8.6 ± 0.3	38 ± 1.4	28.3 ± 1.2
Commercial scale										
25	4000	40	253	276	1200	153	45.9	8.7 ± 0.2	38.3 ± 1.6	30.5 ± 1.4

T: Temperature, ρ_{avg}: Average ginger powder particle size, Q_{CO2}:$SCCO_2$ flow rate, S/F: Solvent to feed ratio

4.3.2.2 Optimization of extraction coupled with online fractionation conditions for production of high-quality products

Supercritical CO_2 fractional separation was coupled with the optimized $SCCO_2$ extraction of ginger for recovery of volatile oil and oleoresin enriched with gingerols in two separators connected in series as per the flowchart, shown in **Fig. 4.4**. Both separators were maintained at different pressures and same optimum temperature (40 ^oC) to prevent degradation of heat labile gingerols and volatile compounds (Nair, 2013). The composition and yield of fractions obtained at different fractionation conditions of separators 1 and 2 have been compared in **Table 4.4**. The results clearly demonstrate the selectivity of $SCCO_2$ for oleoresin and volatile oil fractions at higher and lower operating pressure, respectively. The fractions recovered at separator 1 operating at higher pressure (225, 200, 175 and 150 bar) had a higher content of non-volatile matter (96.2 – 86.1 %)

and lower content of volatile matter (3.9 – 13.9 %). When the pressure decreased from 225 to 175 bar, the wt % of volatile oil in the recovered oleoresin decreased from 5.1 to 3.6 %, resulting in improvement of non-volatile contents from 94.9 to 96.2%. This could possibly be because of increase in the extent of co-precipitation of cuticular waxes owing to the decrease in the solubility of cuticular waxes in the $SCCO_2$-extract mixture. The decreasing pressure also resulted in the increase in quantity of major actives, sum of (6,8,10)-gingerols and 6-shogaol, in oleoresin from 50.6 to 51.2 wt %, since gingerols belong to the family of molecules with higher chain structures. The overall yield was also observed to increase from 3.7 to 6 % of dry ginger taken.

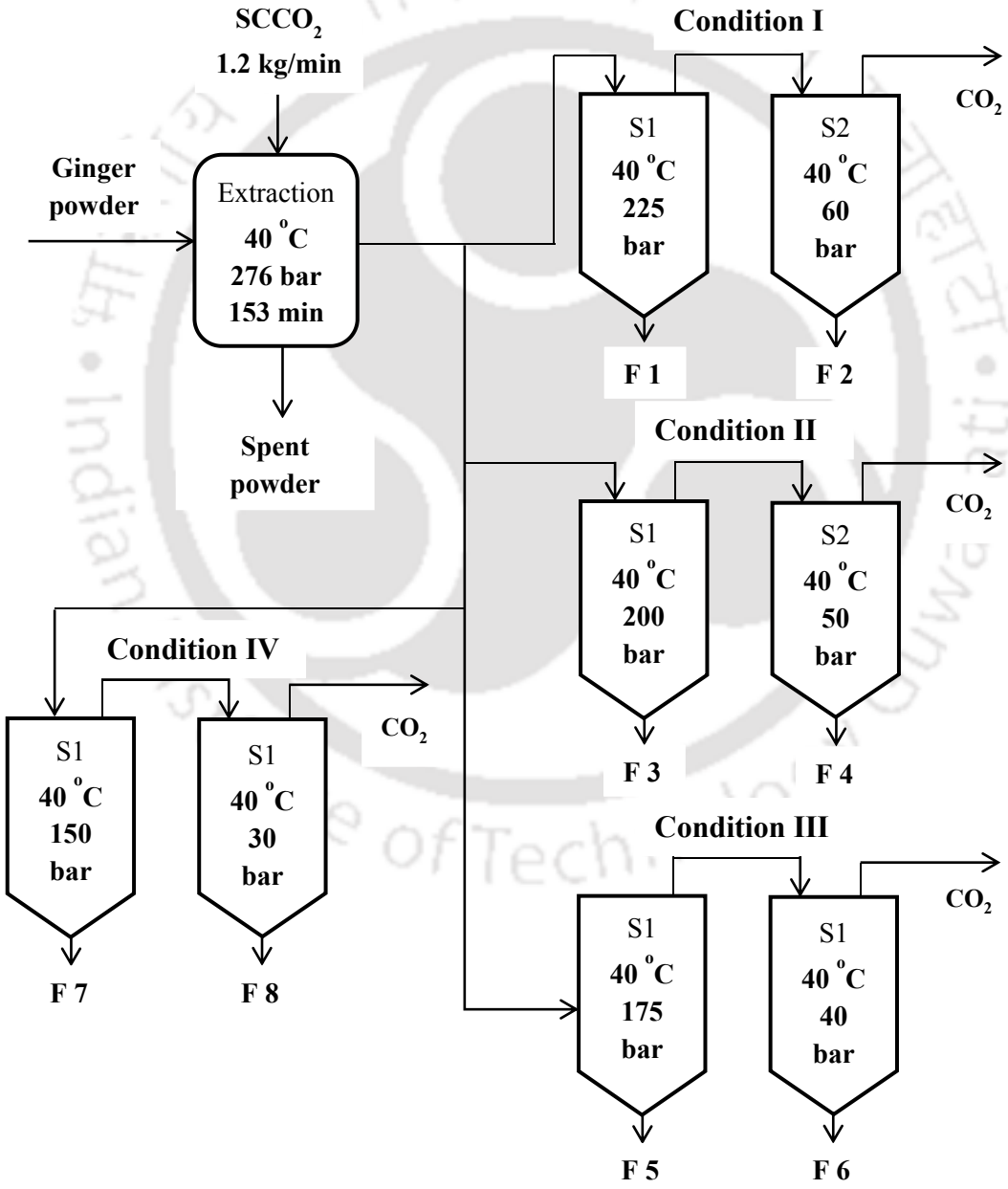


Fig. 4.4: Flow chart of extraction and online fractionation process of ginger powder

TH-2388_13907006

As reported by Da Porto et al. (2014), the decrease in pressure at a constant operating temperature of 40 °C decreases the CO_2 density which results in decreased solvation power and hence solubility of heavier compounds at 225, 200 and 175 bar. Hence, $SCCO_2$ demonstrated the best selectivity for gingerols as well as for non-volatile compounds from a mixture of extract (containing volatile and non-volatile compounds) at 175 bar. Further decrease in pressure to 150 bar, however, changed this trend.

The yield of extract recovered was observed to be highest (7 %) because of substantial co-precipitation of volatile compounds (13.9 wt %) in the oleoresin, consequently decreasing the gingerols content to 45.4 wt %. This could be because of a slight change in $SCCO_2$ selectivity at 150 bar towards volatile compounds. Similar co-extraction of 6-gingerol (up to 20.8 wt %) with volatile fractions has been observed by Salea et al. (2017) at same process conditions of 150 bar/40 °C. The separator 2 was maintained at 40 °C and lower pressure of 60, 50, 40 and 30 bar. At these conditions of temperature and pressure, the CO_2 densities were lower than 600 kg m^{-3}. This caused separation of all volatile components in the extract along with little quantity of non-volatile contents. Because of this reason, the fractions recovered at separator 2 were rich in volatile oil content (72.3 – 96 wt %) and had low non-volatile matter (27.8 – 4 %).

Table 4.4: Quality comparison of recovered fractions in different conditions of extraction coupled with fractionation of dry ginger

Fractionation Conditions	Pressure (bar)		Yield (%)		Major actives@		Volatile oil@	
	1 Sep*	2 Sep	1 Sep	2 Sep	1 Sep	2 Sep	1 Sep	2 Sep
1	225	60	5.3 ± 0.2^{a}	3.4 ± 0.1^{d}	50.6 ± 1.7^{b}	9.5 ± 0.4^{c}	5.1 ± 0.2^{b}	72.3 ± 2.6^{a}
2	200	50	5.5 ± 0.2^{a}	3.2 ± 0.1^{c}	51.1 ± 2.1^{b}	5.7 ± 0.2^{b}	4.9 ± 0.2^{b}	84.3 ± 3.2^{b}
3	175	40	6 ± 0.3^{b}	2.8 ± 0.1^{b}	51.2 ± 2.4^{b}	0.8^{a}	3.6 ± 0.2^{a}	95.9 ± 3^{c}
4	150	30	7 ± 0.2^{c}	1.7 ± 0.1^{a}	42.7 ± 1.6^{a}	0.8^{a}	13.9 ± 0.6^{c}	96 ± 3.9^{c}

different superscript letters within a column represent significant difference at $p<0.05$; @: quantity of major actives and volatile oil expressed in wt % of the total extract recovered; Sep* = separator

The total oil yield ranged from 3.4 – 1.7 % with major actives between 19.5 – 8.9 wt %. The best quality volatile oil separated at 40 and 30 bar which had similar volatile contents of 95.9 and 96 wt %, remaining being non-volatile matters of 4.1 and 4 % respectively. However, the fraction recovered at 40 bar had a higher oil yield of 2.8 % as compared with 1.7 % of that from 30 bar.

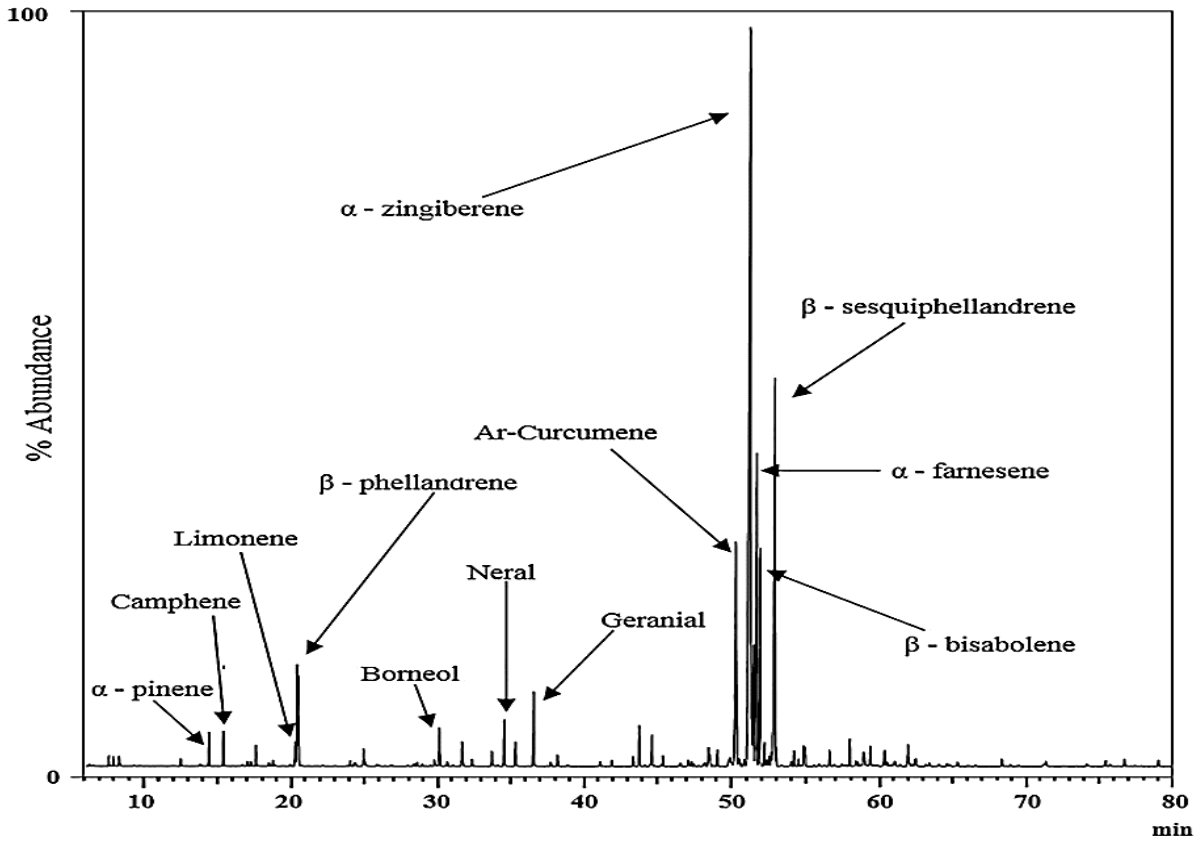


Fig. 4.5: GCMS Chromatogram of $SCCO_2$ fractionated ginger volatile oil

Table 4.5: Quality comparison of $SCCO_2$ fractionated and steam distilled ginger volatile oils

Major Compounds	RRI	Retention time (min)	Content (% area)	
			$SCCO_2$	Steam distilled
α - pinene	939	14.6	0.6	0.16
Camphene	953	15.6	1.7	1.5
Limonene	1030	20.5	0.5	0.6
β - phellandrene	1031	20.7	2.1	1.7
Borneol	1164	30.1	0.8	0.11
Neral	1227	34.6	0.9	0.9
Geranial	1255	36.9	1.7	1.6
Ar-Curcumene	1472	50.1	6.2 ± 0.1	12.9 ± 0.2
α - zingiberene	1487	51.1	37 ± 0.6	24.7 ± 0.6
α - farnesene	1497	51.6	8.9 ± 0.2	7.1 ± 0.2
β - bisabolene	1504	51.8	6.2 ± 0.1	5.9 ± 0.2
β - sesquiphellandrene	1515	52.8	13.6 ± 0.1	6.8 ± 0.1

RRI: Relative retention indices [taken from Raina et al., (2005); Ravi Kiran et al., (2013)]

TH-2388_13907006

Therefore, 40 bar pressure at 40 ^oC was selected as the best condition for recovery of the highest volatile oil yield and quality.

A detailed GCMS graph of thus recovered volatile oil is presented in **Fig. 4.5** and the composition is compared with the steam distilled volatile oil from the same ginger variety in **Table 4.5**. Supercritical CO_2 fractionated volatile oil contained 37 % α – zingiberene and 13.6 % β – sesquiphellandrene (major volatile active compounds) which was higher than the steam distilled oil, hence demonstrating the thermal degradation of these compounds in conventional processing techniques. These volatile actives were also found to be much higher than the reported values of 25.9 and 8.2 %, respectively for $SCCO_2$ extracted volatile oil from a major commercial Australian variety (Bartley and Foley, 1994), thus accounting for high quality of volatile oil produced from $SCCO_2$ fractionation technique.

4.3.3 Comparison of oleoresin extracted with conventional methods

Table 4.6 presents a comparison of oleoresin yield and its 6-gingerol content, extracted with conventional soxhlet method using different solvents vis-à-vis $SCCO_2$ extraction and fractionation methods. The yield of pure fractionated oleoresin was found to be 6 %, which was the lowest when compared with the yields of hexane (6.2 %), 95 % (v/v) ethanol (9.8 %) and acetone (7.6 %). The highest yield of 9.8 % was recorded for soxhlet extraction with 95% ethanol. The same trend was observed for 6-gingerol content, which was 2.2 % for 95 % ethanol as against 0.9 % from hexane and 1.3 % from acetone. This clearly showed higher selectivity of ethanol towards 6-gingerol, which is primarily a phenolic group compound. Ethanol has been very well documented to have excellent selectivity over other solvents for extracting phenolics, antioxidants and gingerols from ginger (Ghasemzadeh et al., 2015; Rahath Kubra et al., 2013).

On the other hand, the recorded yield of 6-gingerol content (on a dry weight basis of ginger) was 2.3 % in $SCCO_2$ extraction, which was highest among all the solvents. Further, the $SCCO_2$ extraction coupled with fractionation was highly selective for 6-gingerol content, causing the enhancement in 6-gingerol's concentration in the recovered oleoresin to 37.4 wt %. In addition, it is noteworthy that all the extracts produced by conventional methods had traces of organic solvents. Thus, it was concluded that $SCCO_2$ extraction coupled with fractionation produced superior quality oleoresin with higher concentration of actives, as compared with conventional methods.

Table 4.6: Comparison of oleoresin extraction with conventional methods

Method	Yield %	6-gingerol (wt % of extract)	6-gingerol (% dry ginger basis)
SCCO$_2$			
Extraction[a]	8.7	26	2.3
Extraction coupled fractionation[b]	6	37.4	2.2
Soxhlet			
Hexane	6.2	14.7	0.9
Ethanol (95 % v/v)	9.8	22.6	2.2
Acetone	7.6	16.8	1.3

a: mixture extract containing volatile oil and oleoresin, b: fractionated pure oleoresin (96.2 wt %)

4.4 Literature comparison

Results of the current study have been compared with the published literature of major ginger varieties around the world in **Table 4.7**. Present study focused on optimizing process parameters primarily for the production of oleoresin enriched with highest quality gingerols. The high quality recovered volatile oil was an added advantage of using the $SCCO_2$ online fractionation technique. The volatile oil yield of the current study was 2.8 %, which was almost similar to that of 2.7 % of $SCCO_2$ extracted volatile oil yield from a Taiwanese variety (Chen et al., 2011), higher than 1.1 % of that from a Thailand variety (Yingngam and Brantner, 2018) and lower than 3.2 % yield of steam distilled volatile oil from a Meghalayan variety (Ravi Kiran et al., 2013). However, α – zingiberene content of 37 %, resulting in the current study, was higher than all the reported values (0.02 to 29.9 %) of **Table 4.7** signifying the high quality of the extracted volatile oil. The oleoresin yield was also found to be higher than most of the reported values of 3.8 – 5 % except for 8.2 % of Varanasi variety which was unfractionated mixture extract (obtained at 250 bar/40 ^oC) and probably contained a lot of other components like volatile oil, waxes etc. The 6-gingerol content of 37.4 wt % in the current study was found to be much higher than all reported values of 18 – 23.8 % (Salea et al., 2017; Tao et al., 2013; Yonei et al., 1995). Such a high concentration of major actives in oleoresin has been achieved due to selective fractionation property of $SCCO_2$ for targeted gingerols, which have been reported for the first time in case of ginger.

TH-2388_13907006

Table 4.7: Comparison of ginger extracts from current study with published literature

Variety	Process details	Equipment details	Optimum process parameters	Product portfolio			Reference
				Type	Yield %	Extract quality parameters	
Laboratory study							
Sungro-sung (India)	$SCCO_2$ extraction	1 extractor (0.5L) & 1 separator (0.5L)	Temp: 40°C, ρ_{avg}: 250 µm, Pressure: 276 bar, Q_{CO2}: 30 g min^{-1}, Time: 153 min	Mixture	8.6	1. 38 wt% major actives (25.6 wt% 6-gingerol); 2. 6-gingerol: 2.2 % (on dry wt basis) 3. 28.3 wt% volatiles	**Current Study**
Varanasi local (India)	Ultrasound assisted $SCCO_2$	1 extractor (0.5L) & 1 separator (0.5L)	Temp: 35°C, ρ_{avg}: 250 µm, Pressure: 250 bar, Q_{CO2}: 15 g min^{-1}, Time: 60 min	Mixture	8.2	N.A.	(Said et al., 2015)
Wonogiri (Indonesia)	$SCCO_2$ extraction	2 extractor (2L each) & 1 separator (N.A.)	Temp: 35°C, ρ < 600 µm, Pressure: 150 bar, Q_{CO2}: 15 g min^{-1}, Time: 160 min	Mixture	3.1	20.7 wt% 6-gingerol 6-gingerol: 0.6 % (on dry wt basis)	(Salea et al., 2017)
Cochin (India)	$SCCO_2$ extraction	1 extractor (0.75L) & 2 separator (0.1L each)	Temp:39.85 °C, ρ <600 µm, Pressure: 195 bar, Q_{CO2}: 15 g min^{-1}, Time: N.A.	Oleoresin	4.0	18.5 wt% 6-gingerol 6-gingerol: 0.7 % (on dry wt basis)	(Yonei et al., 1995)
Pingtun local (Taiwan)	$SCCO_2$ extraction	1 extractor (0.1L)	Temp: 60°C, ρ:< 595 µm, Pressure: 275.8 bar, Q_{CO2}: N.A., Time: 90 min	Volatile oil	2.7	-	(Chen et al., 2011)
Queensland (Australia)	$SCCO_2$ extraction	N.A.	$SCCO_2$ density between 0.680 – 0.935 g ml^{-1}	Volatile oil	N.A.	25.9 wt% α - zingiberene[a]	(Bartley and Foley, 1994)
Meghalaya (India)	Steam Distillation	Clevenger's Type	N.A.	Volatile oil	3.2	100 % pure volatile oil with 29.9 wt% α - zingiberene[a]	(Ravi Kiran et al., 2013)
Cassumunar (Thailand)	Solvent-free microwave extraction (SFME)	Microwave equipped with Clevenger's type apparatus	Microwave power: 700 W, extraction time: 70 min	Volatile oil	1.12	100 % pure volatile oil with 0.02 wt% α - zingiberene[a]	(Yingngam and Brantner, 2018)

a: considering α – zingiberene as a major volatile ingredient for comparison purpose; $SCCO_2$: Supercritical carbon dioxide; Temp: temperature; ρ_{avg}: average ginger powder particle size, Q_{CO2}:$SCCO_2$ flow rate

TH-2388_136107006

Table 4.7: Contd.

Variety	Process details	Equipment details	Optimum process parameters	Product portfolio			Reference
				Type	Yield %	Extract quality parameters	
Tongling White (China)	Ionic liquid based ultrasonic assisted extraction (ILUAE)	Ultrasonic water bath	[C4mim]BF4 conc. :1.5 M, Ultrasound power: 200 W, temp: 25 °C, solid/liquid ratio: 1:20 (g/ml), time:10 min	Mixture	N.A.	6-gingerol: 1 % (on dry wt basis)	(Kou et al., 2018)
China	$SCCO_2$ extraction followed by purification with macroporous resin	N.A.	Temp: 40.9°C, Pressure: 225.9 bar, Time: 265 min	Volatile oil	1.2	46.6 wt% pure volatile oil	(Lei et al., 2016)
Scale-up study							
Sungro-sung (India)	$SCCO_2$ extraction	3 extractor (20L each) & 2 separator (5L each)	Temp: 40°C, ρ_{wg}: 250 µm, Pressure: 276 bar, Q_{CO2}: 30 g min^{-1}, Time: 153 min	Mixture	8.7	1. 38.3wt% major actives (26 wt% 6-gingerol) 2. 6-gingerol yield: 2.2 % (on dry wt basis) 3. 30.5 wt% volatiles	**Current Study**
Wonogiri (Indonesia)	$SCCO_2$	2 extractor (50L each) & 1 separator (N.A.)	Temp: 40°C, ρ_{wg}: 250 µm, Pressure: 276 bar, Q_{CO2}: 30 g min^{-1}, Time: 144 min	Mixture	3.8	18 % 6-gingerol 6-gingerol: 0.7 % (on dry wt basis)	(Salea et al., 2017)
Fractional separation study							
Sungro-sung (India)	$SCCO_2$ extraction coupled with fractionation	3 extractor (20L each) & 2 separator (5L each)	Optimum extraction condition with Separator 1 at 175 bars/40°C (oleoresin recovery) and Separator 2 at 40 bars/40°C (volatile oil)	a. Oleoresin b. Volatile oil	6 2.8	1. 96.2 wt% pure oleoresin with 51.2 wt% major actives (37.4 wt% 6-gingerol) 2. 6-gingerol: 2.2 % (on dry wt basis) 3. 95.9 wt% pure volatile oil with 37 wt% α - zingiberene[a]	**Current Study**

a: considering α – zingiberene as a major volatile ingredient for comparison purpose; $SCCO_2$: Supercritical carbon dioxide; Temp: temperature; ρ_{wg}: average ginger powder particle size, Q_{CO2}:$SCCO_2$ flow rate

4.5 Summary

The chapter presented a method to extract and selectively fractionate gingerols (major actives of ginger) enriched extract from dry ginger along with high quality volatile oil. The conclusions are summarized as follows:

- A single step green process for supercritical CO_2 ($SCCO_2$) extraction coupled with fractionation of dry ginger for simultaneous production of gingerols rich oleoresin and volatile oil has been developed. The optimization and scale-up validation of this process have been achieved in three stages.
- Firstly, $SCCO_2$ extraction conditions were optimized for the highest yield of mixture extract containing a maximum amount of volatile oil components and major actives, expressed in terms of sum of (6+8+10) gingerols and 6-shogaol.
- The optimum process variables under laboratory scale conditions were: average ginger particle size 253 μm, extraction temperature 40 °C, pressure 276 bar, flow rate 30 g min^{-1} and 153 min of extraction time. This resulted in 8.6 % yield of a mixture extract having 38 wt % of major actives and 28.3 wt % of volatile oil.
- In the second stage of experiments, these conditions were scaled up 50 folds on a commercial scale unit. The recovered mixture extract recorded an improvement in 0.1 % yield with an increase of 0.3 and 1.2 wt % in its major actives and volatile oil, respectively.
- Finally, the optimum conditions of online $SCCO_2$ fractionation (coupled with same optimum extraction conditions) resulted in a 6 % oleoresin yield, which was 96.2 % pure and had 51.2 wt % of major actives, in separator 1 operating at 175 bar/40 °C. Simultaneously, 2.7 % yield of volatile oil (95.9 % pure) was recovered in separator 2, operating at 40 bar/40 °C.
- The yield and quality of oleoresin and volatile oil extracted from conventional methods were also compared.

TH-2386_136107006

Chapter 5

Design of a carrier system for gingerols enriched oleoresin tailored for application in water based food product development applications

Work published as:

Shukla, A., Das, C., & Goud, V. V. (2020). Design of a carrier system for gingerols enriched oleoresin tailored for food applications. *Food and Bioproducts Processing*, *124*, 296-306.

TH-2386_136107006

TH-2386_136107006

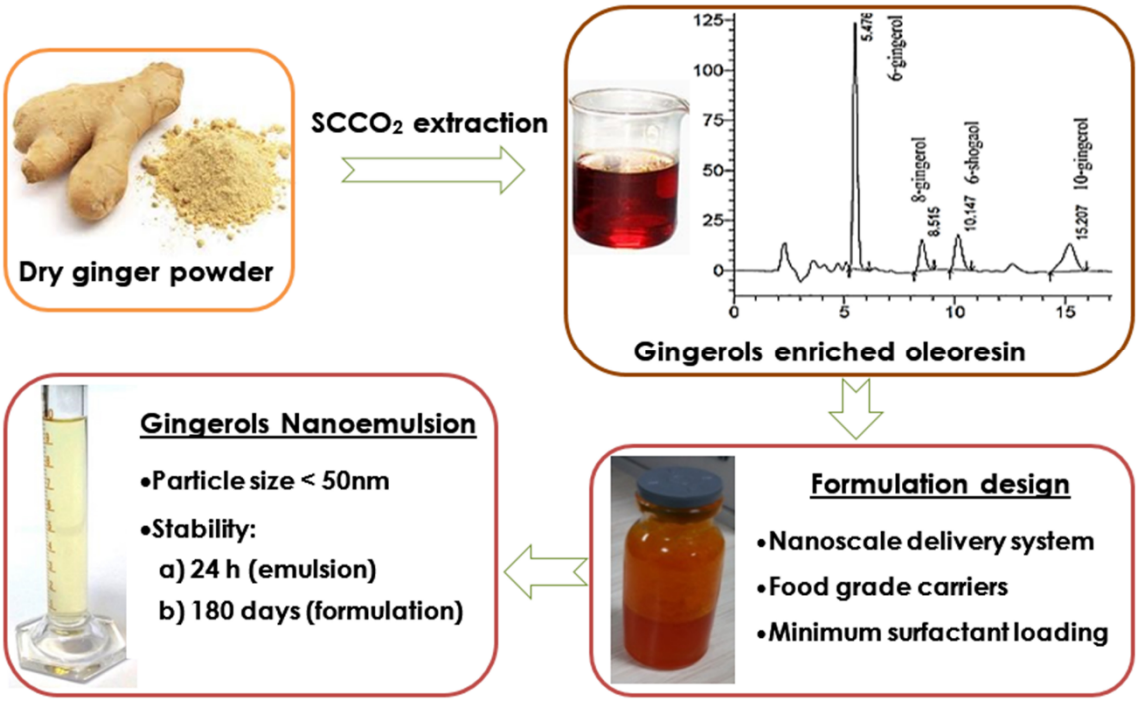
5.1 Background

Food industries employ fortification of additives as a means to impart desired characteristics in food products, which include preferred color, flavor, sensory attributes, and enhancement of shelf-stability. Increasing health concerns have directed the interest of consumer in food products with clean labels and green additives. Hence, plant-based natural extracts are preferred candidates for incorporation in food over synthetic chemicals. These trends have led to exponential increase of market demand fostering new directions and innovative approaches in product development research. Despite the attractive portfolio, the lipidic nature of ginger oleoresin limits its food applications to only oil-based products. Besides, the application in food supplements and the pharmaceutical sector is also highly limited because of poor water solubility of its actives, which severely reduces its oral bioavailability and hence efficacy. Considering the high antioxidant potential of gingerols enriched oleoresin, it is hypothesized that designing a delivery system using food grade excipients with bio enhancing properties for pungent gingerols enriched oleoresin would result in enhanced loading and better water dispersibility as against existing reports. This gingerols rich formulation with low surfactant to oleoresin ratio would be an efficient template for application of ginger actives into various model food systems manufactured in water based processes.

5.2 Overview

Gingerols and shogaols are the major ginger actives present in ginger oleoresin (GO), responsible for distinct pungent flavor. The GO is an ideal natural flavoring agent, however the poor water solubility severely restricts its application for only lipid-based food products. Hence, a spontaneous micro emulsifying carrier system has been designed for enhanced water with a low surfactant to actives (6, 8, 10-gingerols and 6-shogaol) enriched oleoresin ratio, targeted for food fortification applications. A range of food-grade excipients with bio enhancer properties was tested showing Gelucire 44/14, Transcutol 90 and Capryol 90 as best surfactant, co-surfactant and oily vehicle, respectively, for highest water dispersibility of the actives. After that, pseudo ternary diagram study and D-optimal mixture design were employed to find the optimum ratio of all excipients for highest oleoresin loading resulting in dispersion having minimum globule size and maximum emulsification efficiency. The optimized formulation contained 45.5 wt % ginger oleoresin, 27 wt % Gelucire 44/14, 11 wt % Transcutol-90 and 16.5 wt % Capryol-90. It was stable for 90 days in accelerated storage conditions

and its dispersion in water was stable for 24 h with a mean globule size of 47.7 nm, -13.1 mV zeta potential and polydispersity index of 0.22. The in-vitro release study in a wide range of pH confirmed an enhancement of 97.4 % in water dispersibility over pure ginger oleoresin.



Graphical abstract of work presented in this chapter

Indian Institute of Technology Guwahati

TH-2388_136107006

5.3 Results and discussion

5.3.1 Solubility studies

Identification of suitable excipients, namely surfactant, co-surfactant and the oily vehicle having maximal solubilizing potential for gingerols are critical to achieving optimum ginger oleoresin loading (Pouton, 1997). An ideal self-micro emulsifying drug delivery system (SMEDDS) should be isotropic, monophasic and exhibit high solubility to incorporate desired drug loading with minimum carrier volume (Parmar et al., 2011). The excipients hence selected for formulation design consisted of a range of large and medium-chain molecular structure (**Table 5.1**) with HLB values within 13 to 16, ideal for hydrophobic gingerols (Jain et al., 2013). The solubility of gingerols in various excipients selected for the study is presented in **Table 5.1**.

Table 5.1: Solubility of gingerols in various excipients selected for study

Sr. No.	Commercial name	Chemical name	Manufacturer	Type	Solubility (mg g^{-1})
1.	Gelucire 44/14	Lauroyl polyoxyl-32 glycerides	Gatteffose, France	Surfactant	140.9 ± 4.3
2.	Kolliphor T 80 (Tween 80)	Polyoxyethylene (20) sorbitan monooleate	BASF, Germany	Surfactant	61 ± 2.1
3.	Kolliphor RH EL	Macrogolglycerolricinoleate	BASF, Germany	Surfactant	29.3 ± 0.9
4.	Kolliphor RH 40	Macrogol glycerolhydroxystearate	BASF, Germany	Surfactant	33 ± 1.8
5.	Kolliphor RH 15	Polyoxyl 15 Hydroxystearate	BASF, Germany	Surfactant	32.6 ± 0.9
6.	Kolliphor PG	Propylene Glycol (PG)	BASF, Germany	Co-surfactant	5.5 ± 0.2
8.	Kolliphor PEG 400	Poly Ethylene Glycol 400 (PEG 400)	BASF, Germany	Co-surfactant	42.2 ± 1.5
9.	Transcutol HP	Diethylene Glycol Monoethyl Ether	Gatteffose, France	Co-surfactant	67.7 ± 3.5
10.	Capryol 90	Propylene Glycol Monocaprylate	Gatteffose, France	Oily Vehicle	68.4 ± 2.9
11.	Lauroglycol 90	Propylene Glycol Monolaurate	Gatteffose, France	Oily Vehicle	17.1 ± 0.4
12.	Labrafac Lipophile	Medium-chain Triglycerides	Gatteffose, France	Oily Vehicle	5.4 ± 0.1

Amongst the various oily phases selected for study, Capryol 90 gave maximum solubility of target gingerols (68.4 mg g^{-1}). On the other hand, the surfactants that gave maximum solubility was Gelucire 44/14 (140.9 mg g^{-1}) followed by Tween 80 (61 mg g^{-1}) along with the co-surfactant, Transcutol 90 (67.7 mg g^{-1}). The selection of surfactant or co-surfactant was further governed by their emulsification efficiency, which is enumerated in terms of percentage (%) transmittance values, rather than just their ability to solubilize ginger oleoresin (Date and Nagarsenker, 2007). The % transmittance values of dispersions in the water of ginger oleoresin mixed with various excipients are presented in **Table 5.2(a)**. Emulsification studies clearly distinguished the ability of various surfactants to emulsify ginger oleoresin (GO). It indicated a significant difference in emulsification efficiencies of selected surfactants with best candidate being Gelucire 44/14 having 93.5 % ability to emulsify GO followed by Tween 80 with 75.7 % and least value of 58.3 % for Kolliphor RH EL. The result corresponds to the findings of Warisnoicharoen et al. (2000), who concluded that emulsification ability is influenced by the structure and chain length of the surfactant.

Table 5.2: (a) Emulsification efficiency of various non-ionic surfactants, and (b) Emulsification studies on surfactant/co-surfactant combinations

(a)

Surfactant	% transmittance
Gelucire 44/14	93.5 ± 2.6
Tween 80	75.7 ± 1.1
Kolliphor RH 15	64.3 ± 1.6
Kolliphor RH 40	60.1 ± 2.2
Kolliphor RH EL	58.3 ± 2.9

(b)

Co-surfactant	Gelucire 44/14	Tween 80
Trancutol 90	96.9 ± 1.3	93.9 ± 1.5
PEG 400	89.9 ± 2.7	88.8 ± 3.5
PG	84.8 ± 2.7	86.3 ± 2.6

Gelucire 44/14 and Tween 80 rendered very fine emulsions requiring a very short time and were selected for further investigations for selecting best co-surfactant. The

results presented in **Table 5.2(b)** clearly distinguished the ability of various co-surfactants in further improving the emulsification ability of Gelucire 44/14 and Tween 80. All selected co-surfactants enhanced the ease of the emulsion formation. Here again, a good correlation was observed between the structure and chain length of co-surfactant (or molecular volume) and the transmittance values of resulting dispersions. Larger molecular volume of the co-surfactants resulted in lesser transmittance values, as presented in **Table 5.2(b)**. The co-surfactant acts as a second amphiphile and plays a vital role in reducing the overall interfacial tension by penetrating the interfacial surfactant monolayer. This results in reducing mechanical barrier, which leads to coalescence and ultimately leading to improvement in emulsification ability of surfactants (Parmar et al., 2011). Out of all the combinations studied, Transcutol 90 with Gelucire 44/14 presented highest emulsification efficiency of 96.9 % and was hence selected for further studies.

5.3.2 Pseudo-ternary phase diagrams

Based on previous screening studies, Gelucire 44/14 was selected as the surfactant, Transcutol 90, as co-surfactant and Capryol 90 as oily vehicle. Pseudo-ternary phase diagram study was conducted in order to precisely locate the optimum formulation ratios resulting in most efficient clear emulsion zones from coarse emulsion and phase separation zones. Ginger Oleoresin (GO) was mixed with oily vehicles in various ratios, namely, 4:1 (GO mix-1), 3:1 (GO mix-2), 2:1 (GO mix-3), 1:1 (GO mix-4), pure oleoresin (GO mix-5) and 1:2 (GO mix-6). As per the recommendations of Date and Nagarsenker (2007), various formulations of ternary systems were made in which surfactant, co-surfactant and GO mixes were varied from 25 to 75 wt %, such that the sum of all components added together to 100 %.

The formulation which formed the maximum desired region of transparent one phase emulsion was one with a 3:1 ratio of GO with Capryol 90 as shown in **Fig 5.1(b)**. The effect of increase in the percentage of Capryol 90 in GO mixtures had an inverse effect on the desired region for 2:1, 1:1 and even for pure ginger oleoresin which can be seen in **Fig. 5.1(c-e)**, respectively. In **Fig. 5.1(a)**, a crossover effect could be seen where the increase in GO content started to reduce the desired region. The observed crossover effect could be because an optimum surfactant to oil ratio exists which makes best water in oil emulsion. Further increase in oil content results into decreased efficiency of oil-in oil emulsion formation. Hence, based on **Fig 5.1(b)**, GO mixed with Capryol 90 in ratio

of 3:1 was considered optimum, and the percentage range of excipients giving the best emulsion was chosen as shown in **Table 5.3** for further optimization studies.

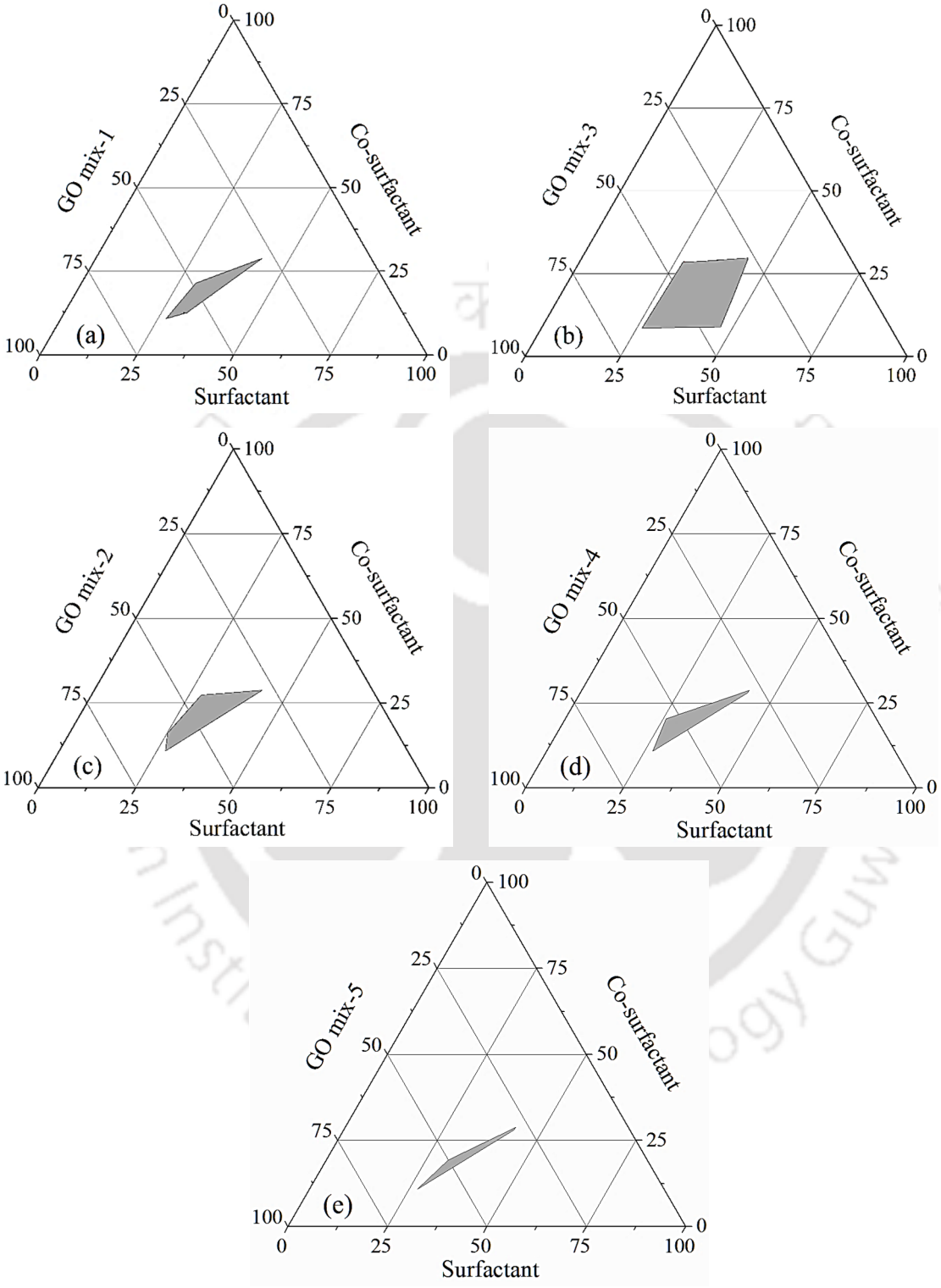


Fig. 5.1: Pseudo ternary phase diagrams of various formulations of surfactant, co-surfactants and oil mixtures with ratio of ginger oleoresin (GO): Capryol 90 being 4:1 as GO mix-1(a), 3:1 as GO mix-3 (b), 2:1 as GO mix-2 (c), 1:1 as GO mix-4 (d) and pure ginger oleoresin as GO mix-5 (e); shaded region corresponds to clear one phase emulsion in water

TH-2388_136107006

Table 5.3: Selected ranges of excipients for optimization of formulation

Excipient	Minimum (wt %)	Maximum (wt %)
Surfactant (Gelucire 44/14)	27	43
Co-surfactant (Transcutol 90)	11	30
Oil (3:1 mixture of Ginger oleoresin:Capryol 90)	28	62

5.3.3 Optimization of formulation using the design of experiments

Based on the results of pseudo-ternary diagram studies, further optimization studies were conducted for finding the optimum ratio of excipient, resulting in the best SMEDDS formulation.

Table 5.4: Experimental design matrix with actual data

Run	Uncoded values of independent variables			Responses	
	Surfactant (%)	Co-surfactant (%)	GO mixture (%)	Globule size (nm)	% Transmittance
1	43	22.7	34.3	94.4	90.1
2	43	12.3	44.7	37.7	96.9
3	35.7	30	34.4	150.7	81.6
4	27	29.2	43.8	161.7	78.7
5	27	11	62	65.4	98.6
6	33.6	19	47.4	92.8	85.6
7	27.3	17.8	54.9	100.8	85.7
8	35.1	11	53.9	44.7	96.5
9	42.4	29.6	28	136.1	87.9
10	39.5	19.3	41.3	94.1	88.7
11	27.2	23.4	49.3	122.5	81.3
12	35.1	11	53.9	42.3	94.1
13	43	12.3	44.7	36.1	94.1
14	27	11.00	62	48.1	95.6
15	27	29.2	43.8	165.2	81
16	42.4	29.6	28	138.8	84

D-optimal mixture design was employed on the selected range of excipients (**Table 5.3**) resulting into 16 experimental runs. Three independent variables were: surfactant (A), co-surfactant (B) and GO mixture (C) with two experimental response results, globule size (Y_1) and % transmittance (Y_2). The experimental results are shown in **Table 5.4**.

For the selection of a mathematical model that best described the experimental data, various available models were analysed for lowest values of predicted residual sum of square statistics. The following cubic model (equation 5.1) was selected for independent variable Y_1 (globule size, nm) and quadratic model (equation 5.2) for Y_2 (% transmittance).

$$Y_1 = -649.83 \times A + 613.29 \times B + 64.64 \times C + 1225.61 \times A \times C - 747.38 \times B \times C - 416.4 \times A \times B \times C + 1514.61 \times A \times B \times (A - B) + 774.43 \times A \times C \times (A - C) - 510 \times B \times C \times (B - C) \quad (5.1)$$

$$Y_2 = 116.32 \times A + 83.4 \times B + 96.78 \times C - 46.99 \times A \times B - 33.69 \times A \times C - 41.87 \times B \times C \quad (5.2)$$

The *p*-value of the above equations was less than 0.05, showing the significance of the model, as presented in the ANOVA analysis (**Table 5.5**).

Table 5.5: Analysis of variance (ANOVA) for the fitted linear model

Factor	Globule size (nm)						% Transmittance				
	SS	*df*	MS	F	p		SS	*df*	MS	F	p
Model	30676.9	9	3408.5	1273.52	< 0.0001	Model	618.01	5	123.60	39.78	< 0.0001
Linear Mixture	30439.2	2	15219.6	5686.46	< 0.0001	Linear Mixture	550.4	2	275.2	88.59	< 0.0001
AB	7.41	1	7.4	2.7	0.1471	AB	9.69	1	9.69	3.12	0.1079
AC	57.36	1	57.3	21.4	0.0036	AC	9.59	1	9.59	3.09	0.1094
ABC	76.50	1	76.50	28.58	0.0018	BC	27.20	1	27.20	8.76	0.0143
AB(A-B)	2.51	1	2.51	0.94	0.3703	-					
AC(A-C)	163.8	1	163.8	61.23	0.0002	-					
BC(B-A)	56.6	1	56.6	21.16	0.0037	-					
Residual	74.8	1	74.8	27.97	0.0018	Residual	31.07	10	3.11		
Lack-of-Fit	16.06	6	2.68			Lack-of-Fit	11.59	5	2.32	0.59	0.7087
Pure Error	3.99	1	3.99	1.65	0.2551	Pure Error	19.48	5	3.90		
R^2	99.6 %					R^2	95.2 %				
R^2 (adjusted)	99.9 %					R^2 (adjusted)	92.8 %				
R^2 (predicted)	98.4 %					R^2 (predicted)	87.4 %				

TH-2388_136107006

Fig. 5.2 graphically presents the variation of fitted experimental data of surfactant (A), co-surfactant (B), and GO mixture (C) as functions of independent variables (Y_1 and Y_2). The percentage transmittance (78.7 to 98.6 %) and globule size (36.1 to 164.5 nm) significantly increased and decreased, respectively, by increasing surfactant content and reducing co-surfactant content. It implied that accommodation of more amounts of either surfactant or co-surfactant is essential for a stable interfacial film around ginger oleoresin globules resulting in clear and transparent formation of nanoemulsion. It was because more amounts of surfactant reduced interfacial tension between ginger oleoresin and water interface, which plays a vital role in reducing globule size (Date and Nagarsenker, 2007; Joshi et al., 2013). On the contrary, lowest globule size (<50nm) was found for formulations having lower contents of surfactant (27-30 wt %) and co-surfactants (11-13 wt %) implying better formation of emulsion at such concentrations. This could because, for a specific system, an optimum limit of co-surfactant exists when used in combination with another surfactant for yielding the best emulsification. Beyond this limit, additional co-surfactant results in a crossover effect, which reduced the ease of spontaneous emulsification (Pouton, 1997, 2000).

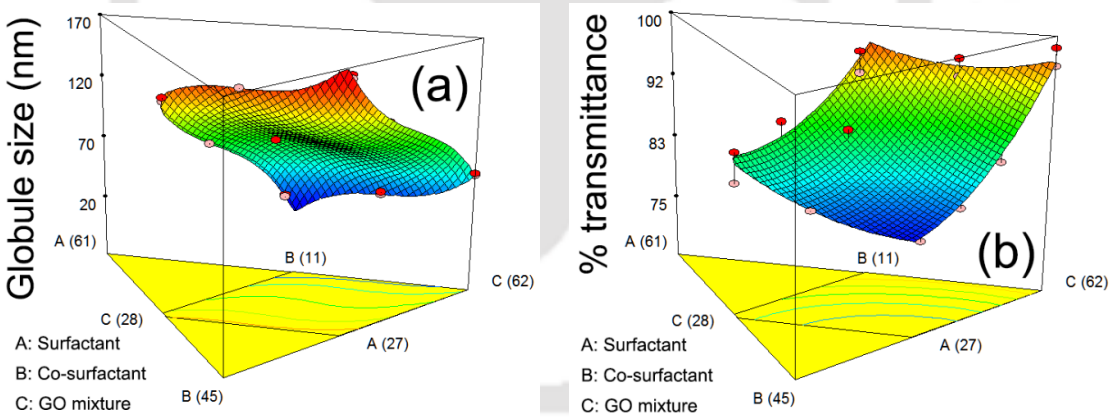


Fig. 5.2: Response plots of variation in (a) globule size (nm) and (b) % transmission

5.3.3.1 Numerical optimization

The optimization of developed formulations for minimum globule size and maximum % transmittance and was performed by Design-Expert software's desirability function for maximum desirability. It was also desired to have surfactant to oil (GO mixture) ratio as low as possible for our specific food application requirement.

Table 5.6: Comparison of predicted and experimental values of optimized formulations

Surfactant wt %	Co-surfactant wt %	GO mixture wt %	Globule size (nm)	% Transmittance
Predicted				
27	11	62	48.8	95.6
Experimental				
27	11	62	47.7 ± 1.1	98.7 ± 0.9

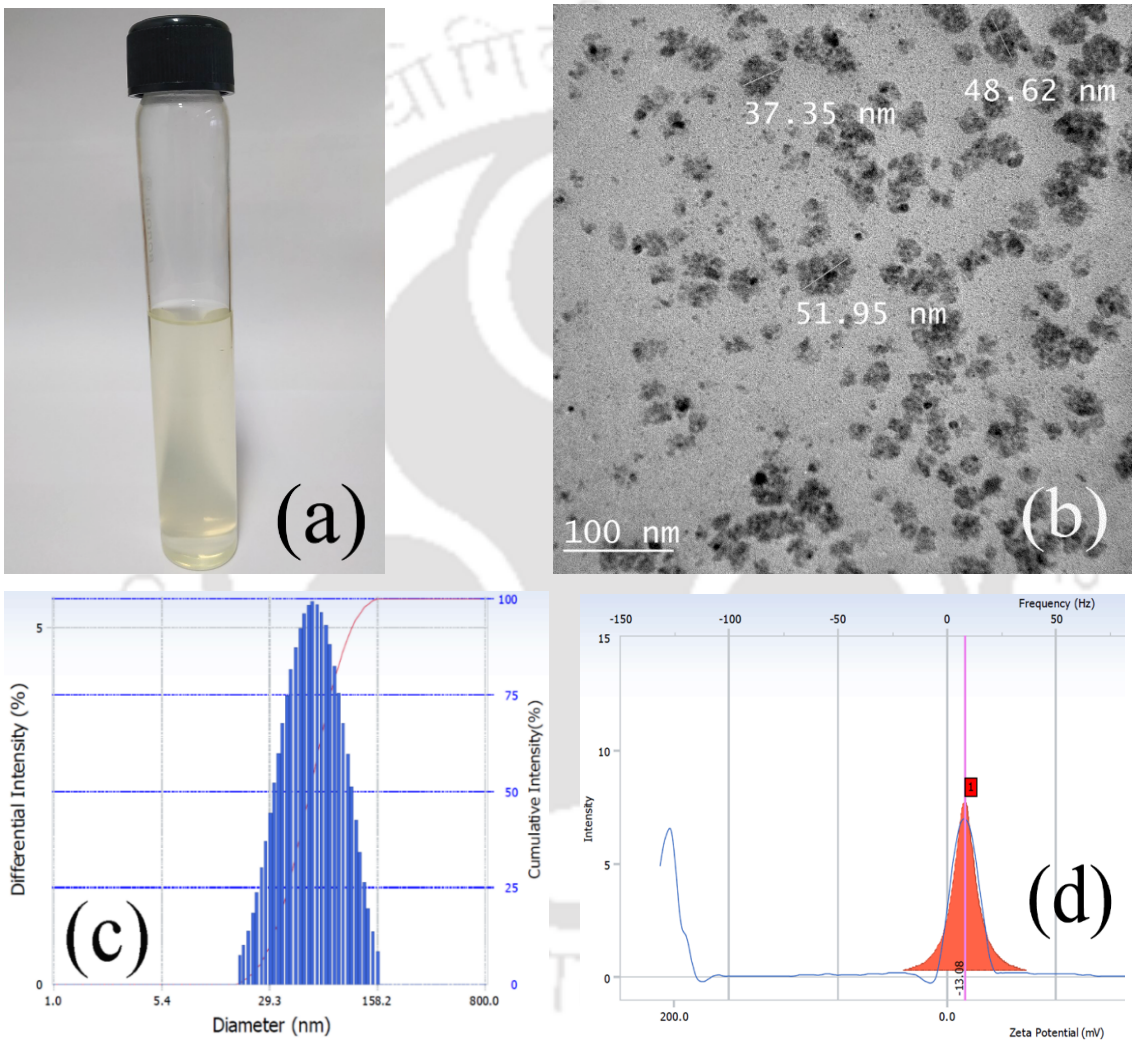


Fig. 5.3: Globule size characteristics of nanoemulsion prepared from optimized formulation. (a) Snap of nanoemulsion, (b) TEM image of nanoemulsion, (c) Average particle size result obtained from photon correlation spectroscopy, and (d) result of its zeta potential

TH-2388_13107006

Hence out of all the suggested predictions, formula with 27 wt % surfactant, 11 wt % co-surfactant, and 62 wt % GO mixture having desirability value of 0.91 was chosen and was prepared in triplicate, characterized and compared with model predicted values, as presented in **Table 5.6**.

A snap of emulsion made from the developed formulation is presented in **Fig. 5.3(a)**. A transmission electron microscopy (TEM) image showing particle size of the nanoemulsion is presented in **Fig. 5.3(b)**. Average globule size result obtained from photon correlation spectroscopy was 47.7 nm, as presented in **Fig. 5.3(c)** and the result of its zeta potential is shown in **Fig. 5.3(c)**, which was found to be -13.1 mV. The actual experimental values of % transmittance and globule sizes were 98.7 % and 47.7 nm, respectively which were within a 3 % deviation from model predicted values of 95.6 % and 48.8 nm, respectively, suggesting high accuracy of the developed model. The optimized formulation also showed polydispersity index of 0.22, which was considerably low thus representing narrow size distribution of globules within the nanoemulsion. Diluting the nanoemulsion to 100 times result in dispersed globules to remain in nano range. This showed affinity to more quantity of aqueous media. Thus, it was concluded that D-optimal based mixture design was suited for optimizing ginger oleoresin based formulation with desired characteristics. The blend of 27 wt % surfactant (Gelucire 44/14), 11 wt % co-surfactant (Transcutol HP), and 62 wt % of ginger oleoresin mixture with Capryol 90 in 3:1 ratio was finalized as optimized formulation.

5.3.4 Characterization of optimized ginger oleoresin loaded SMEDDS

5.3.4.1 Robustness to dilution

The dilution test is necessary to understand the in-vivo stability of dispersion made from the developed formulation. No sign of creaming or phase separation was observed after 50, 100 and 1000 times dilutions, and all the dispersions continued to be transparent one phase systems at the end of 48 h.

5.3.4.2 In vitro release behaviour

The study of pH behaviour is significant for formulations designed for the oral route because the absorption of the active drug takes place in a range of different pH mediums existing in different parts of the human digestive mechanism route. The effect of pH on in-vitro release behaviour of the optimized formulation has been compared with

that of pure ginger oleoresin in **Fig. 5.4**. The gingerols of pure oleoresin exhibited very poor dissolution irrespective of pH and time because the gingerols are known to be hydrophobic due to their lipid nature (Bhattarai et al., 2001). On the contrary, the developed formulation showed remarkable enhancement in dissolution of gingerols, which was 95.4 %, 79.8 %, and 83.9 % higher than pure oleoresin at pH 1.2, 7, and 7.4, respectively. At low pH, -OH group of gingerol could have facilitated the association with the solvent or excipient/co-excipient for enhancing the solubility. The mixture of surfactant, co-surfactant and oily phase designed as carrier and optimized for ginger oleoresin encapsulated the hydrophobic gingerols. These microcapsules, when mixed with water, spontaneously formed micelles and very efficiently dispersed the formulation into very fine droplets. No precipitation or agglomeration of the oleoresin was observed, and the resultant was practically a one phase transparent system.

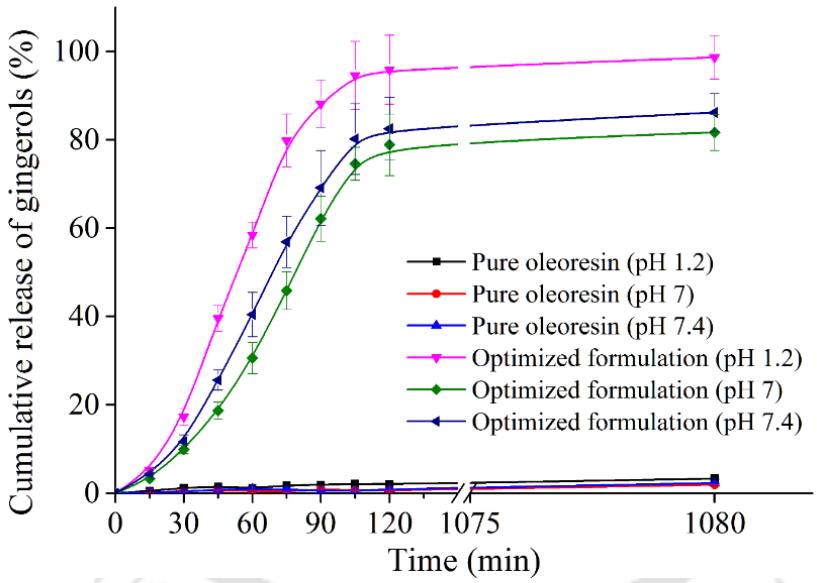


Fig. 5.4: Comparison of in-vitro release profiles of pure ginger oleoresin and optimized formulation at different pH mediums

5.3.4.3 Stability of optimized formulation

The short term stability of developed formulation's dispersion is critical to understand in the release, and absorption behavior during metabolism as well as for designing dosage. Besides, knowledge of long term shelf-storability behavior of the developed formulation is imperative for designing packaging applications (Rezaei et al., 2019).

The result of short-term storage stability study of the dispersion prepared from the optimized formulation presented no precipitation or color variations up to 12 h (**Fig.**

TH-2388_136107006

5.5). The zeta potential values (in mV) were nearly constant from -13.1 mV at zero h to -11.6 mV at the end of 24 h but decreased abruptly to -5.9 mV at 36 h and -3.6 mV by the end of 48 h. This trend is also seen in the percentage (%) transmittance values, which remain relatively constant from 98 – 96 % from 0 to 24 h and after that started to fall abruptly. Based on these values, it can be clearly said that the dispersion prepared from optimized formulation is stable up to 24th h of storage and after that, the ginger oleoresin particles start to agglomerate. This behaviour is ideal for immediate release drugs and especially suits the specific requirements of food applications wherein the entire absorption gets completed within a maximum of 6 h (Kuentz, 2011).

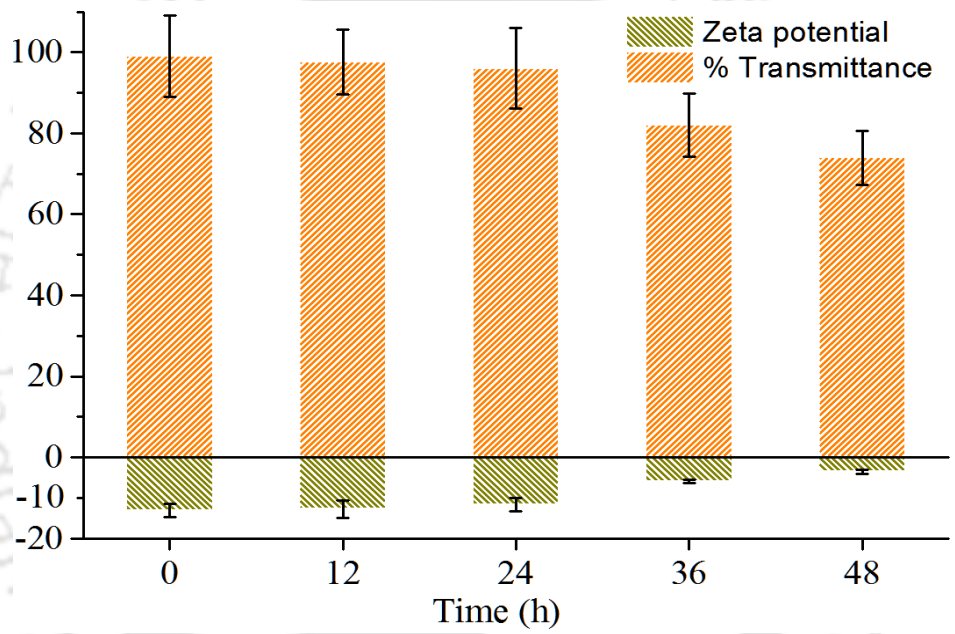


Fig. 5.5: Zeta potential and % transmittance values of the dispersion prepared from optimized formulation during short term storage

Table 5.7 gives a summary of various characteristic parameters of the optimized formulation after long-term storage of 90 days at 40 ^{o}C and 75 % RH. For the case of formulation, the retention of gingerols was 96.5 %, implying minimal degradation of actives. The encapsulation provided by the carrier molecules possibly prevented the temperature-sensitive gingerols from being degraded to shogaols. The dispersion prepared from the stored formulation was also found to be clear one phase system with minimal changes in the particle size, zeta potential and % transmittance values as against that of freshly prepared values. From the results of the storage study it could be concluded

that the developed formulation could be stable under normal storage conditions for at least six months.

Table 5.7: Long term (90 days) storage stability parameters of optimized formulation

Characteristic Property	Value
Retention of gingerols	96.5 ± 2.4 %
Globule size	59.6 ± 1.1 nm
Zeta Potential	-10.2 mV
% transmittance	94.4 ± 0.2

5.4 Comparison with published literature

The present work has been compared with the results of a few available studies attempting the development of carrier systems for the delivery of ginger actives, as presented in **Table 5.8**. The wt % of actives loading in formulation developed in the current work was 23.8 % (14.9 % 6-gingerol, 2.8 % 8-gingerol, 4.5 % 10-gingerol and 1.6 % 6-shogaol). It was highest among the published literature range of 1.4 to 7.3 %, even for the case of formulations using only major active ingredient, thus resulting in efficient formulation with substantially low surfactant to oleoresin ratio. The particle size in the dispersion prepared from the formulation was also found to be minimum, i.e. 47.7 nm for current study, as against 73 nm to 85.3 µm of the literature. The zeta potential of water dispersion prepared from optimized formulation of current study was found to be -13 mV. It was comparable to -13.1 mV for the case of phytosome prepared from HPLC grade 6-gingerol (Singh et al., 2018) and higher than -2.5 mV for solid lipid nanoparticles (SLNs) of 6-gingerols (Xu et al., 2016).

Wang et al. (2018) reported SLNs for a 98.7 % pure 6-shogaol extract, resulting in zeta potential -15.2 mV. On the other hand, water dispersibility enhancement in the current study was also found to be highest except for the case of SLNs of ginger oleoresin, which was 300 % as reported by Ogino et al. (2018). Both these works achieved higher zeta potential and water dispersibility owing to use of substantially higher amounts of surfactants resulting in considerable decrease in actives loading of 5.9 wt % (Wang et al., 2018) and 1.5 wt % (Ogino et al., 2018), respectively. For food applications, such high surfactant loadings may not be desirable owing to regulatory considerations (Additives and Food, 2015).

TH-2388_136107006

TH-2386_136107006

Table 5.8: Comparison of the current study with published reports on similar ginger formulations

Sr. No.	Ginger material used	Formulation portfolio	Formulation characteristics	Achievements	Reference
1	$SCCO_2$ extracted ginger oleoresin with 52.2 wt% of actives [(6+8+10) gingerols and 6-shogaol]	Method: SNEDDS Excipients: Gelucire 44/14, Transcutol 90 and Capryol 90	Particle size: 47.7 nm Zeta potential: -13 mV	Actives loading: 23.8 wt% Enhancement of (a) 95.4 % solubility and (b) anti-cancer potential	Current study
2	Solvent extracted and purified 6-shogaol extract with 98.65 wt% purity	Method: Solid Lipid Nanoparticles Excipients: Medium-chain triglycerides, glyceryl monostearate, tween 80 and span 80	Particle size: 73.6 nm, Zeta potential: -15.2 mV,	Actives loading: 5.9 wt% Enhancement of (a) 29 % solubility and (b) anti-gout activity	(Wang et al., 2018)
3	Solvent extracted and purified 6-gingerol extract with 75.34 wt% purity	Method: SMEDDS Excipients: Ethyl oleate, Cremophor EL35 and 1,2-Propanediol	Particle size: 73.1 nm, Zeta potential: -2.5 mV,	Actives loading: 6.4 wt% Enhancement of (a) 70 % solubility and (b) oral bioavailability	(Xu et al., 2016)
4	$SCCO_2$ extracted ginger oleoresin with 30 wt% of actives [(6+8) gingerols and 6-shogaol]	Method: S-SEDDS Excipients: Medium-chain triglycerides, Lysolecithin, Glycerin and hydroxypropyl methylcellulose	Particle size: 114 nm, Zeta potential: N.A.	Actives loading: 1.5 wt% Enhancement of (a) 3 folds solubility and (b) pharmacokinetic behavior	(Ogino et al., 2018)
5	HPLC grade 6-gingerol with 97 wt% purity	Method: Phytosome complexed with chitosan Excipients: Soya lecithin and chitosan	Particle size: 254 nm, Zeta potential: -13.1 mV	Actives loading: 7.3 wt% Enhancement of anti-bacterial activity	(Singh et al., 2018)
6	Ginger oleoresin with 0.14 wt% of actives (6-gingerol + 6-shogaol)	Method: Spray chilled solid lipid microparticles Excipients: Palmitic acid, oleic acid or palm fat	Particle size: 85.3 µm, Zeta potential: N.A.	Actives loading: 1.4 wt% N.A.	(Oriani et al., 2016)

SNEDDS: Self nano-emulsifying drug delivery system, SMEDDS: Self micro-emulsifying drug delivery system, S-SEDDS: Solid self-emulsifying drug delivery system

TH-2388_126107006

Table 5.8: Contd.

Sr. No.	Non-ginger based material	Formulation portfolio	Formulation characteristics	Achievements	Reference
1.	Cefpodoxime Proxitil	Method: SNEDDS Excipients: Cremophore EL, Akoline MCM, and Capryol 90	Particle size: 150-170 nm, Zeta potential: N.A.	Actives loading: 20 wt% Enhancement of 100 % solubility	(Date & Nagarsenker, 2007)
2.	Lutein	Method: SNEDDS Excipients: Lexol, Emulmetik 900, Labrasol and Tween 80	Particle size: 40-150 nm, Zeta potential: -19 to -32 mV	Actives loading: 0.5 wt% Enhancement of 90 % solubility	(Niamprem et al., 2014)
3.	Wheat germ oil	Method: Nanoemulsion prepared through sonication Excipients: Maltodextrin, Tween 20	Particle size: 114.7 nm, Zeta potential: 14.76 mV	Actives loading: 3.6 wt% Enhancement of color, sensory and delay in oil oxidation during cold storage of cooked mackerel fillet	(Ceylan et al., 2019)
4.	Thyme oil	Method: Nanoemulsion prepared through sonication Excipients: Maltodextrin, Tween 20	Particle size: 163 nm, Zeta potential: -24.8 mV	Actives loading: 5.5 wt% Anti-microbial effect during cold storage of fish fillet	(Meral et al., 2019)

SNEDDS: Self nano-emulsifying drug delivery system

Hence, minimum surfactant to oleoresin ratio achieved in the current study is most suited for food applications. Moreover, comparison with results of various researchers dealing with delivery design for other (non-ginger) actives also supports the effectiveness of carrier design targeted for nano scale delivery of ginger actives.

5.5 Summary

The present work attempted to develop a stable formulation of ginger oleoresin for the delivery of lipophilic ginger actives targeted for food applications. Specific objectives achieved are:

- The optimized formulation contained 27 wt % surfactant (Gelucire 44/14), 11 wt % co-surfactant (Transcutol 90), 46.5 wt % of ginger oleoresin, and 15.5 wt % Capryol 90 as oily vehicle.
- The dispersion prepared from the formulation had globule size in nano range with average globule size 47.7 nm was and stable upto 24 h.
- The anhydrous formulation was stable in long term storage for 90 days in accelerated conditions (40 ^{o}C and 75 % RH).
- The developed formulation also showed 95.4 % enhancement in water dispersibility of ginger actives over pure oleoresin.
- Thus, it was concluded that the developed formulation with food-grade carriers for the delivery of ginger actives of ginger oleoresin has a promising commercial potential, especially in the food industries.

TH-2386_136107006

Chapter 6

Gingerols infusion for development of mango fruit based ready to eat nutraceutical food product

Work published at:

Shukla, A., Shukla, R. S., Das, C., & Goud, V. V. (2019). Gingerols infusion and multi-step process optimization for enhancement of color, sensory and functional profiles of candied mango. *Food Chemistry, 300*, 125195.

TH-2386_136107006

TH-2386_136107006

6.1 Background

Mango, king of all the fruits, is popular for its unique taste, aroma and high contents of β-carotene and vitamin C. Owing to its distinct flavor profile mango finds its way into a wide range of culinary as well as processed food applications across the globe. Candying is one of the most important preservation techniques that result in a value addition and substantial improvement in the shelf life of mango. Increasing health awareness has recently directed the focus of research in food fortification with natural extracts for further enhancement of functional and nutraceutical properties. Many researchers have demonstrated substantial improvement in the functional and shelf qualities of final products by employing the osmotic-assisted infusion of water-soluble bioactive compounds like anthocyanin in the watermelon rinds. This chapter deals with the incorporation of water dispersible formulation of gingerols enriched ginger oleoresin (covered in previous chapter) into mango fruit matrix for developing a functionally rich food product. The present work hypothesizes that incorporation of pungent gingerols in candied mango will synergistically enhance the unique sweet-sour flavor palette of mango along with improving its nutraceutical properties. Till date, no systematic reports are available which involve either gingerols infusion or optimization of the candying process for the minimum loss of mango's natural bioactive compounds (vitamin C, β-carotene, etc.). In addition, reports on the choice of drying methods suitable in large manufacturing scale settings for minimum degradation of the functional and nutritive quality of candied mango are also scanty. Moreover, process scale-up studies involving a comparison of fundamental engineering properties like mass transfer coefficients, diffusion coefficients, equilibrium contents and product's quality parameters in terms of chemical and sensory properties as well as batch production time, with bench scale data, give critical insights in making important choices like feasibility comparison with existing methods, process economics etc. Such studies ultimately help in the commercialization of newer candying techniques and very limited literature is available in this area.

6.2 Overview

The presence of pungent gingerols in ginger oleoresin makes it an ideal natural flavoring candidate for the food industry. The study reports its incorporation for synergistic enhancement of flavor and nutraceutical portfolio of candied mango. The process is systematically optimized at a bench-scale for gingerols infusion and subsequent

candying treatment in a range of hypo and hypertonic osmotic solutions for critical transport properties. After that, optimization of the drying process and the scale-up study was conducted with a 200-fold increase in the batch size. Mass transfer and quality parameters were comparable in both scales, implying excellent repeatability and scalability of the process. Infusion of gingerols led to significant improvement in sensory scores of the candied product when compared with scores of fresh mangoes and non-infused product. Multistep process optimization also resulted in improving the retention of critical quality parameters of β-carotene, total phenolics, and vitamin C, to 85.6, 76.8 and 60.2 % values of fresh mango contents, respectively, along with minor color difference. When compared with a commercial candied mango product, manufactured under similar conditions, vitamin C, total phenolics, and β-carotene contents enhanced by 233.3, 131.8 and 42.7 %, respectively, along with superior color, sensory and textural properties. Moreover, 376.7% reduction in overall processing time was also achieved in product manufactured by optimized process.

Graphical abstract of the work presented in this chapter

TH-2388_136127006

6.3 Results and discussion

6.3.1 Bench-scale experiments

6.3.1.1 Optimization of gingerols dispersion strength for the infusion of gingerols into mango fruit slices

It was hypothesized that ginger's unique pungent flavor will go along very well with the sweet-sour flavor palette of mango and this flavour synergy can be successfully exploited in developing a ginger flavoured candied mango product. Hence the first step in developing the product was preliminary optimization studies for fixing the amount of gingerols to be infused into fresh mango fruit matrix. An optimum amount of gingerols infused in fresh mango matrix is critical for producing candied mango product having desirable sensory qualities. **Fig. 6.1** presents an industrial process for mango candy production.

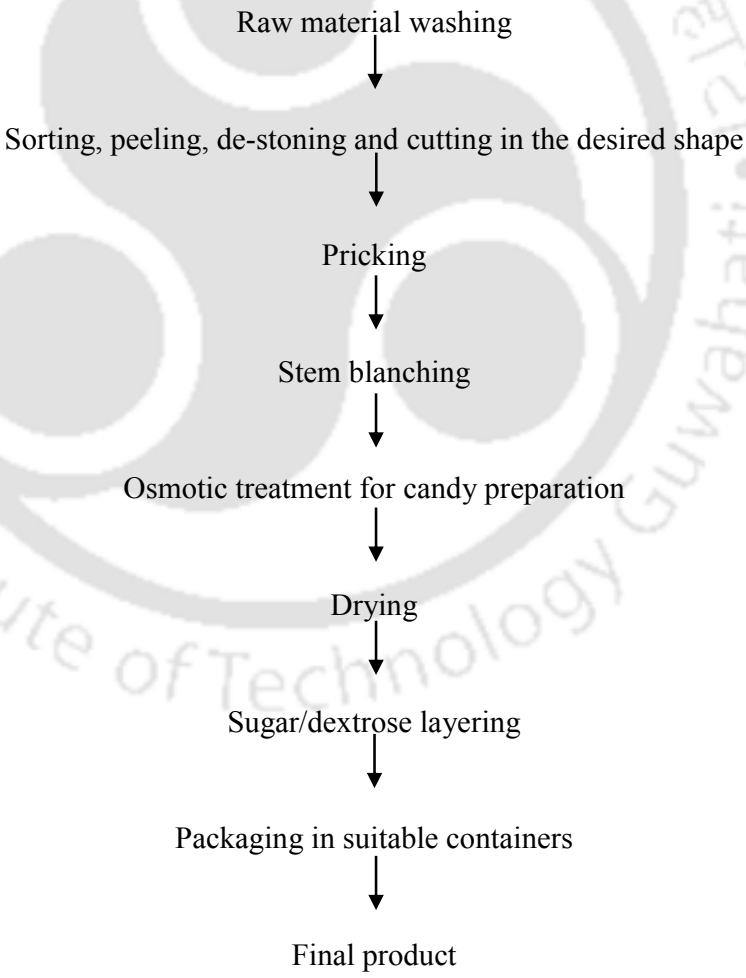


Fig. 6.1: Industrial process of mango candy production

The pre-processing steps involved are washing, sorting, peeling, cutting mango fruit into desired shapes, and pricking the slices. Thereafter, the mango slices were treated in gingerols dispersion to facilitate gingerols infusion into mango fruit matrix. The gingerols infused mango slices were subjected to subsequent processing steps for producing candied mango product according to **Fig. 6.1**.

For gingerols treatment, various water dispersions were prepared using the developed formulation (as discussed in previous chapter) in a concentration range of 0.1 to 10 wt %. The fresh mango slices after pre-processing steps were treated in each water dispersion and candied mango was prepared. The sensory score profiles of candied mango products prepared in each solution are presented in **Fig. 6.2**.

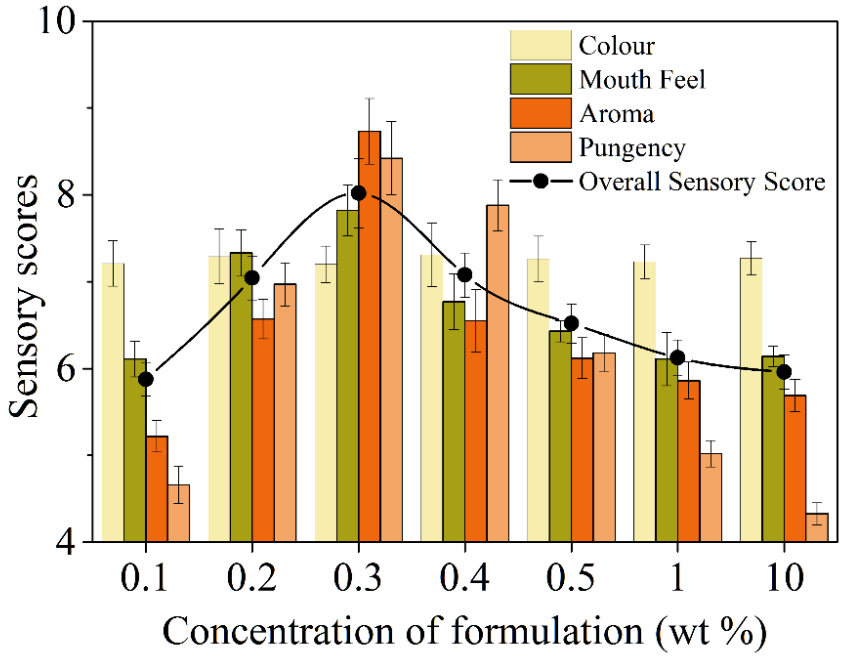


Fig. 6.2: Sensory score profiles of candied mango prepared from mango slices treated in different concentrations of developed ginger oleoresin loaded formulation

Amongst all concentrations, products treated in 0.3 wt % resulted in highest quality in terms of all sensory parameters, namely color, mouthfeel, aroma, and pungency, resulting in highest overall sensory score (*OSS*) value of 8. Most significant effect was observed in scores of aroma, followed by mouth feel and then pungency. Increase in the concentration of formulation did not show any significant change in the score of color. Treatments below the concentration of 0.3 wt % could not impart sufficiently detectable ginger flavor to the product, resulting in lower overall sensory

TH-2388_136107006

scores. Treatments above 0.3 wt % concentration of formulation resulted in pungency dominating the overall flavor spectrum of candied mango product, thereby decreasing the sensory scores.

6.3.1.2 Optimization of infusion of gingerols into mango fruit slices

Osmotic assisted infusion of bioactives into food matrix is one of the easiest and most effective means of incorporating gingerols into mango fruit matrix. Hence, further optimization studies were carried out to fix the strength and duration of osmotic assisted treatment. Osmotic solutions of different strengths (0, 7.5, 15 and 25 ^{o}Brix) were prepared in water with sugar as osmotic agent and 0.3 wt % of the developed gingerols formulation for treating mango slices (after pre-processing steps, **Fig. 6.1**).

Fig. 6.3(a)-(c) shows the effect of osmotic solutions with increasing strength of sugar (0, 7.5, 15 and 25 ^{o}Brix) on the rate of infusion of gingerols, and solids and moisture transfer for the treated mango slices. The magnitude of osmotic pressure exerted by the solution is directly proportional to the concentration of solutes present in it (Perry and Green, 1999). Owing to the presence of mango's natural solutes, the osmotic pressure existing inside the mango cells was much higher than the osmotic pressure of surrounding 0 ^{o}Brix solution (negligible solutes). In order to minimize this osmotic gradient, water diffused into the mango cells along with the gingerols, as gingerols are water-dispersible and did not contribute significantly to the osmotic pressure, as presented in **Fig. 6.3(a)**. The extent of this osmotic gradient decreased with an increase in the concentration of surrounding solution up to 15 ^{o}Brix, thus reducing the rate and magnitude of mass transfer coefficients (**Table 6.1**), compared with osmotic gradient of 0 ^{o}Brix. This also reflected in decrease of effective diffusion coefficients of moisture gain (D_{ew}) from 2.29 $\times$ 10^{-9} m^2s^{-1} to 1.54 $\times$ 10^{-9} m^2s^{-1}, solids loss (D_{es}) from 2.53 $\times$ 10^{-9} m^2s^{-1} to 1.22 $\times$ 10^{-9} m^2s^{-1} and gingerols gain (D_{eg}) from 3.57 $\times$ 10^{-9} m^2s^{-1} to 1.51 $\times$ 10^{-9} m^2s^{-1} (**Table 6.1**). At this concentration, the osmotic pressure inside the mango cells was almost equal to that of the surrounding medium, because the change in solids and moisture contents of mango slices were minimal with respect to time, as shown in **Fig. 6.3(b) and (c)**. An increase in the concentration to 25 ^{o}Brix resulted in a further increase in osmotic pressure of the solution which led to the reversal of the direction of mass transfer of solids and moisture (**Fig. 6.3(b) and (c))**, whereas, gingerols continued to infuse into the mango slices. This concentration resulted in dewatering and simultaneous impregnation of sugar into mango

slices. The highest infusion of 4.4 mg gingerols 100 g^{-1} resulted from immersing slices for 5 h in 0 °Brix (**Fig. 6.3(a)**). Moisture, solids, and gingerols mass transfer, as well as diffusion coefficients, were also found to be highest in case of 0 °Brix (**Table 6.1**). This implied that 0 °Brix was responsible for maximum infusion of gingerols into mango matrix.

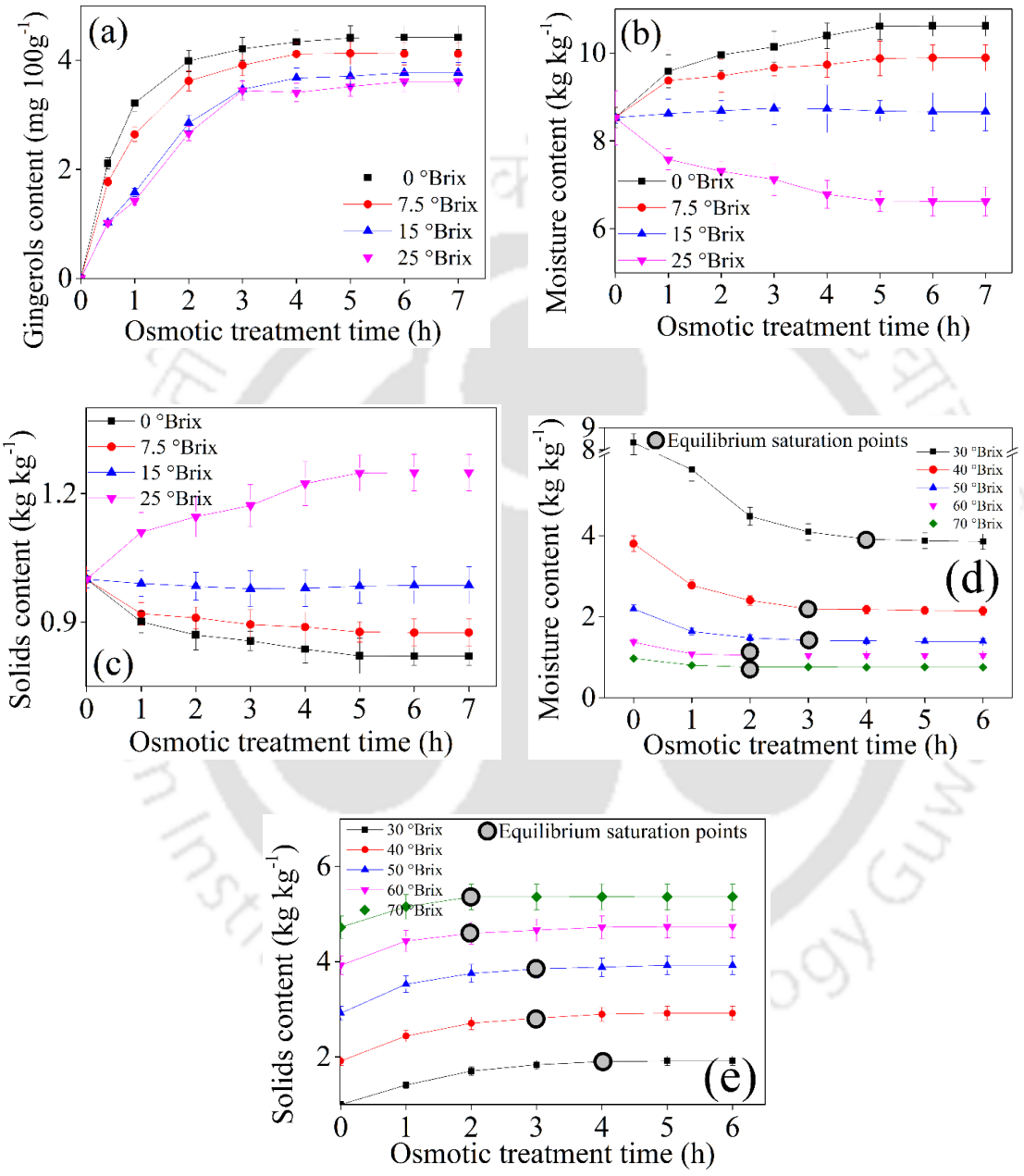


Fig. 6.3: Bench-scale experiments of mango slices for (a) infusion of gingerols, (b) change of moisture content during infusion, (c) change of solids content during infusion, equilibrium saturation of (d) moisture content during candying and (e) solids content during candying process in various strengths of sugar solutions.

TH-2388_136107006

Table 6.1: Diffusion coefficients of gingerols, solids and moisture contents during infusion and candying process in bench and commercial scale experiments

Concentration (°B)	Mass transfer coefficient (k_w, k_s or k_g, h^{-1})			Diffusion coefficient (D_{ew}, D_{es} or D_{eg} x 10^{-9}, m^2 s^{-1})			Equilibrium content ($x_{\infty w}$ or $x_{\infty s}$, kg kg^{-1} and $x_{\infty g}$, mg $100g^{-1}$)		
A. Bench-scale experiments									
Infusion process	*Moisture*	*Solids*	*Gingerols*	*Moisture*	*Solids*	*Gingerols*	*Moisture*	*Solids*	*Gingerols*
0	0.82 ± 0.03^{d}	0.90 ± 0.02^{d}	1.27 ± 0.03^{c}	2.29 ± 0.04^{d}	2.53 ± 0.02^{d}	3.57 ± 0.03^{c}	9.83 ± 0.21^{c}	0.88 ± 0.02^{b}	4.35 ± 0.03^{d}
7.5	0.67 ± 0.02^{c}	0.71 ± 0.01^{c}	1.05 ± 0.02^{b}	1.87 ± 0.04^{c}	1.99 ± 0.04^{c}	2.95 ± 0.04^{b}	8.70 ± 0.22^{b}	0.82 ± 0.01^{a}	4.10 ± 0.02^{c}
15	0.55 ± 0.02^{a}	0.44 ± 0.01^{a}	0.54 ± 0.02^{a}	1.54 ± 0.02^{a}	1.22 ± 0.03^{a}	1.51 ± 0.01^{a}	6.58 ± 0.14^{a}	1.26 ± 0.03^{d}	3.81 ± 0.02^{a}
25	0.60 ± 0.01^{b}	0.67 ± 0.03^{b}	0.56 ± 0.01^{a}	1.68 ± 0.03^{b}	1.89 ± 0.02^{b}	1.56 ± 0.01^{a}	5.62 ± 0.11^{d}	0.98 ± 0.02^{c}	3.97 ± 0.03^{b}
Candying process	*Moisture*		*Solids*	*Moisture*		*Solids*	*Moisture*		*Solids*
30	0.86 ± 0.03^{a}		0.56 ± 0.03^{a}	2.41 ± 0.03^{a}		1.56 ± 0.03^{a}	3.80 ± 0.01^{a}		1.99 ± 0.01^{a}
40	0.90 ± 0.02^{b}		0.68 ± 0.02^{b}	2.53 ± 0.03^{b}		1.92 ± 0.01^{b}	1.54 ± 0.03^{b}		2.94 ± 0.01^{b}
50	1.03 ± 0.03^{c}		0.87 ± 0.03^{c}	2.88 ± 0.01^{c}		2.43 ± 0.03^{c}	1.38 ± 0.03^{c}		3.92 ± 0.03^{c}
60	1.08 ± 0.02^{d}		0.90 ± 0.03^{d}	3.03 ± 0.03^{d}		2.53 ± 0.02^{d}	0.98 ± 0.01^{d}		4.73 ± 0.03^{d}
70	1.10 ± 0.01^{d}		0.92 ± 0.01^{d}	3.08 ± 0.03^{d}		2.58 ± 0.03^{d}	0.74 ± 0.03^{e}		5.37 ± 0.03^{e}
B. Commercial scale experiments									
Infusion process	*Moisture*	*Solids*	*Gingerols*	*Moisture*	*Solids*	*Gingerols*	*Moisture*	*Solids*	*Gingerols*
0	0.49 ± 0.03	0.56 ± 0.02	1.25 ± 0.03	1.37 ± 0.04	1.57 ± 0.02	3.51 ± 0.03	10.70 ± 0.21	0.81 ± 0.02	4.13 ± 0.03
Candying process	*Moisture*		*Solids*	*Moisture*		*Solids*	*Moisture*		*Solids*
30	0.81 ± 0.03^{c}		0.63 ± 0.01^{a}	2.26 ± 0.01^{c}		1.76 ± 0.03^{a}	3.71 ± 0.03^{a}		2.08 ± 0.02^{a}
40	0.82 ± 0.01^{d}		0.64 ± 0.03^{c}	2.31 ± 0.02^{d}		1.79 ± 0.02^{c}	1.82 ± 0.01^{b}		3.04 ± 0.03^{b}
50	0.83 ± 0.02^{b}		0.68 ± 0.03^{b}	2.33 ± 0.02^{b}		1.89 ± 0.03^{b}	1.10 ± 0.03^{c}		4.12 ± 0.02^{c}
60	0.95 ± 0.03^{a}		0.78 ± 0.03^{d}	2.65 ± 0.03^{a}		2.19 ± 0.03^{d}	0.90 ± 0.01^{d}		4.95 ± 0.03^{d}
70	0.95 ± 0.02^{d}		0.86 ± 0.02^{d}	2.66 ± 0.01^{d}		2.42 ± 0.03^{d}	0.61 ± 0.03^{e}		5.57 ± 0.01^{e}

different letters within a column show statistical significance at $p < 0.05$

The results of infusion treatments, obtained in this study have been compared with the other reported literature and found to follow a similar trend for eg. the highest infusion of grape phenolics in model food system (Rózek et al., 2010), anthocyanins in *Amla* (Adsare et al., 2016), apple (George et al., 2016) and curcuminoids in coconut slices (Bellary and Rastogi, 2012), each in 0 °Brix solution.

6.3.1.3 Optimization of the candying process

The gingerols infused mango slices were further subjected to candying process in osmotic solutions of 30-70 °Brix containing water-dispersible gingerols formulation at 0.3 wt% concentration (having gingerols concentration of 300 mg L^{-1}). The high osmotic pressure of the surrounding solution around the mango slices resulted in osmotic dehydration (OD) and simultaneous impregnation of sugar. For 30 °Brix, this simultaneous increase of solid content and dehydration of samples was observed until 4 h of treatment (**Fig. 6.3(d) and (e)**). Further increase in the duration of immersion showed insignificant ($p< 0.05$) increase or decrease in solid or moisture contents. This implies that the equilibrium saturation state of solids and moisture transport was achieved at the fourth hour of the treatment. At this condition, the diffusion coefficients for moisture and solids contents were found to be 2.41×10^{-9} m^2s^{-1} and 1.56×10^{-9} m^2s^{-1}, respectively (**Table 6.1**).

Increasing osmotic solution strength to 40 and 50 °Brix resulted in the equilibrium at 3 h, which further reduced to 2 h for both 60 and 70 °Brix (**Fig. 6.3(d) and (e)**). The complete candying process led to the dehydration of mango slices from an initial moisture content of 8.33 kg water kg dry $matter^{-1}$ to a final equilibrium moisture content of 0.74 kg water kg dry $matter^{-1}$ at the end of 70 °Brix. It also resulted in simultaneous impregnation of sugar from 1.99 kg solids kg dry $matter^{-1}$ to 5.37 kg solids kg dry $matter^{-1}$ (**Table 6.1**).

The commercial manufacturing process involves immersing the mango slices for a minimum 24 h each in osmotic solutions of 30, 40, 50, 60 and 70 °Brix, making the total candying time to 5-7 days. However, based on the equilibrium saturation conditions of solids and moisture transport within mango slices, the whole process was achieved in just 4, 3, 3, 2 and 2 h of immersion time in osmotic solutions of 30, 40, 50, 60 and 70 °Brix, respectively. This implied that the immersion of mango pieces in the osmotic solutions for any further duration would only result in leaching of vitamin C and other

TH-2388_136107006

natural solutes of mango into osmotic solution. Hence, these equilibrium points are optimum for minimum processing loss of natural functional ingredients of mango.

Mass transfer and diffusion coefficients of moisture and solids of mango slices increased with an increase in the concentration of osmotic solution (**Table 6.1**). This implies that the extent of OD and sugar impregnation improves with an increase in the osmotic concentration. Considering the equilibrium saturation points as optimum treatment time in each osmotic solution, the sum of total time for complete candying process was 14 h. This was significantly less from total candying time of 20 h, for a reported bench scale study on *Amla* candy (Adsare et al., 2016). This could be due to difference in the morphological characteristics, porosity, and textural strengths between *Amla* and mango tissues.

6.3.2 Scale-up studies

6.3.2.1 Gingerols infusion and candying process

Scale-up studies are essential for bringing a product from development stages at bench scale, to its commercialization stage (Shukla et al., 2019b). The optimum parameters of gingerols infusion and candying process obtained at bench scale were scaled-up 200-fold to produce 100 kg batches of candied mango slices at a commercial scale facility. As per **Fig. 6.4(a)**, maximum gingerols infusion was found to be 4.1 mg $100g^{-1}$ at 5^{th} h. Beyond this time, no significant change (p<0.05) in gingerols, moisture, and solids content was observed in mango slices (**Fig. 6.4(a)**). The infused gingerols content was 6.8 % less than that achieved at the bench scale (4.4 mg $100g^{-1}$). Mass transfer (k_g) and effective diffusion coefficient of gingerols (D_{eg}) into the mango slices were 1.25 h^{-1} and 3.51×10^{-9} m^2 s^{-1}, which were comparable to 1.27 h^{-1} and 3.57×10^{-9} m^2 s^{-1} of bench scale coefficients, respectively (**Table 6.1**). **Fig. 6.4(b)** represents the equilibrium saturation achieved at different stages of the candying process, conducted at a commercial scale facility. No significant changes (p<0.05) in the solid and moisture contents of gingerols infused mango slices were observed beyond 5, 4, 3, 2, and 2 h treatment time in 30, 40, 50, 60 and 70 °Brix sugar solutions, respectively. The total candying time under scale-up condition was 16 h, which was 14.3 % higher when compared with 14 h of total candying time of bench scale condition.

Mass transfer coefficients of moisture (k_m) and solids (k_s) into gingerols infused mango slices increased from 0.81 h^{-1} to 0.95 h^{-1} and 0.63 h^{-1} to 0.86 h^{-1}, respectively during the candying process in osmotic solutions of 30 to 70 °Brix. These were slightly

lower than the bench scale moisture, and solids mass transfer coefficient range of 0.86 h^{-1} to 1.1 h^{-1} and 0.56 h^{-1} to 0.92 h^{-1}, respectively. Similarly, the diffusion coefficients of moisture (D_{ew}) and solids (D_{es}) increased with an increase in the concentration of osmotic solutions (**Table 6.1**) and were slightly lower than the bench scale values. The scale-up effect attributed to a slight difference in the driving force for solid and moisture transport. Owing to this reason, a deviation was observed in the measured transport properties of gingerols, sugar and water of candied mango slices, when compared with bench scale values. This extent of deviation was within a range of 15 %. Various researchers have reported a deviation of upto 25 % in critical process parameters when scaling the process from bench to pilot and manufacturing scales due to scale up effects (Chen et al., 2018; Tolve et al., 2018).

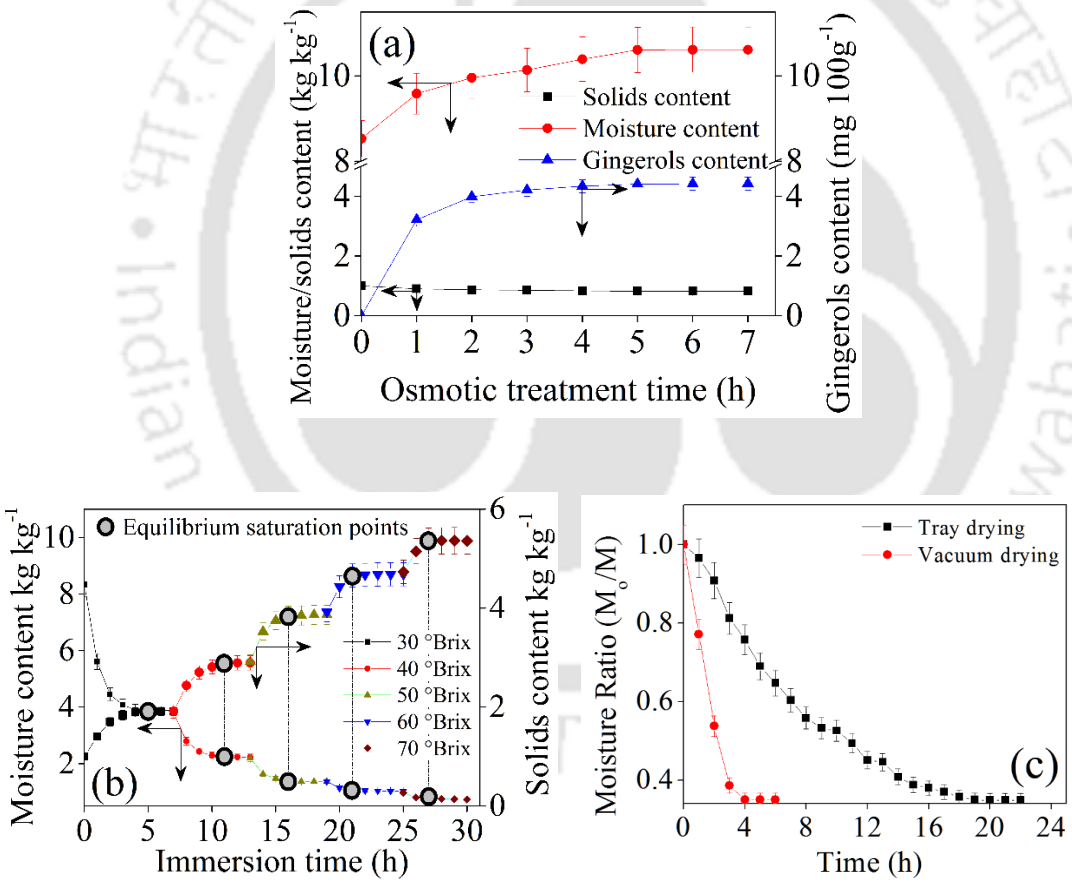


Fig. 6.4: Scale-up experiments for (a) infusion of gingerols, (b) candying process and (c) drying process

However, there was an overall agreement in the extent of OD and sugar impregnation behaviour between bench and manufacturing scale, which increased with

TH-2388_13307006

increase in the concentration of osmotic solutions during the candying process. This implies excellent repeatability and scalability of the process, which is critical for successful process scale-up.

6.3.2.2 Drying characteristics

After the completion of the candying process, gingerols infused mango slices showed a layer of osmotic solution adhering to the slices. Hence, for removal of this surficial moisture, the candied samples were dried in a commercial scale tray and vacuum dryers, respectively. The reduction in the moisture from an initial content of 0.61 kg water kg dry matter^{-1} (at the end of the candying process) to a final content of about 0.15 kg water kg dry matter^{-1} was achieved. The drying parameters chosen for both commercial scale dryers were: conventional tray dryer operating at 80 ^{o}C and vacuum dryer operating at 60 ^{o}C, having a vacuum of 90 KPa. These parameters were chosen based on the commercial scale drying process optimized for maximum retention of vitamin C for candied slices of other similar fruit matrices.

Fig. 6.4(c) presents the drying curves of the gingerols infused mango slices in both the dryers. The drying rates were found to be 1.46 × 10^{-5} kg water. kg dry matter^{-1} h^{-1}, for tray dryer and 4.86 × 10^{-5} kg water. kg dry matter^{-1} h^{-1} for vacuum dryer (**Table 6.2**).

Table 6.2: Moisture diffusivities and drying rates for commercial-scale drying of mango candy slices

Drying method	Drying time*, h	Moisture diffusion coefficient (D_e × 10^{-11}, m^{2}s^{-1})	Drying rate (k × 10^{-5}, kg water. kg dry matter^{-1}h^{-1})	Vitamin C degradation (%)
Tray drying	20 ± 0.5	1.83 ± 0.05	1.46 ± 0.03	23.5 ± 1.1
Vacuum drying	6 ± 0.1	18.78 ± 0.75	4.86 ± 0.17	3.3 ± 0.2

*final moisture content of product = 13.5 % and water activity (a_w = 0.4)

The presence of vacuum reduced the boiling point of water in the vacuum dryer, resulting in faster moisture removal rates from the sample slices as compared to conventional dryer (Telfser and Galindo, 2019). This also resulted in a ten-fold increase in the effective moisture diffusion coefficients (D_e) from 1.83 × 10^{-11} m^2 s^{-1} of tray dryer

to 18.78 × 10^{-11} m^2 s^{-1} for vacuum dryer (**Table 6.2**). The calculated D_e values were within the general range of 10^{-9} to 10^{-11} m^2 s^{-1} for drying of food materials (Madamba et al., 1996) and in line with the reported values of 0.1 × 10^{-11} m^2 s^{-1} to 7 × 10^{-11} m^2 s^{-1}, for air drying of candied mango slices (Giraldo et al., 2005).

In addition to faster drying rates in lower temperatures, the extent of vitamin C degradation in vacuum dried samples was found to be 3.3 % compared to 23.5 % when compared to initial values, in samples dried using tray dryer (**Table 6.2**). Hence, for high product quality, commercial scale vacuum drying proved to be the better option. This observation is in line with the result of various researchers demonstrating advantages of bench-scale vacuum dryer over conventional dryer for high product quality for a variety of food materials (Krumreich et al., 2018; Link et al., 2017). In addition, lower initial cost coupled with operational simplicity and substantially low energy requirements of vacuum drying systems makes it economically attractive means of drying, especially in manufacturing scale settings.

6.3.3 Effect of optimum process parameters on quality characteristics of products

6.3.3.1 Vitamin C and total phenolic content (TPC)

Candied mango was produced with the combination of optimized parameters viz. gingerols infusion, candying, and drying treatments, as described in the previous sections. Five batches each of 0.5 kg and 200 kg were made at bench and manufacturing scales, respectively. **Table 6.3(a)** compares the quality characteristics of these final products with fresh mango used for the study. Average vitamin C contents of candied mango samples were 147.8 and 145.2 mg $100g^{-1}$ for bench and manufacturing scales, respectively, as against 241.1 mg 100 g^{-1} of fresh mango on a dry weight basis. Vitamin C is water-soluble phytochemical, which is responsible for the antioxidant activity of mango. It tends to leach out when mango slices are immersed in the osmotic solutions (Adsare et al., 2016). Because of this, the retention of vitamin C was 61.3 % and 60.2 %, respectively in bench and manufacturing scale samples. The deviation of only 1.8 % in vitamin C contents of both scales implied excellent scalability and repeatability of the process.

A quantitative measure of TPC (water-soluble components) also gives an idea about the antioxidant potential of a sample (Shukla et al., 2019a). Average values of TPC were observed to be 54.9 and 52.1 mg GAE $100g^{-1}$ for both scales as against 67.8 mg GAE $100g^{-1}$ of fresh mango.

TH-2388_13137006

Retention of TPC for bench and manufacturing scales samples was 81 and 76.8 %, respectively to that of values observed with fresh mango. High TPC values with a deviation of only 5.3 % within both scales supported the vitamin C results.

6.3.3.2 Color characteristics

Color characteristics are very important in assessing the effects of processing on fruits. As per Silva and Silva (1999), a typical range of ΔE (total color difference) values can be categorized as per the following criteria: value upto 0.2 for negligible difference, 0.2 to 6 as minor, 6 to 12 as major difference, and values greater than 12 indicate substantially high color difference. Bench and manufacturing scale products showed a minor difference of 4.4 and 5.2, respectively in ΔE values **(Table 6.3(a))**.

These values only give a qualitative idea of the total difference in the color from a reference value, and conclusive inference of good or bad effect cannot be drawn (Chakraborty et al., 2016; Kaushik et al., 2014).

Therefore, BI (browning index) was calculated for the better understanding of the effects of processing on the color characteristics of the produced samples. This represents browning of the samples, and lower values are desirable in terms of consumer acceptability of the product.

Bench and manufacturing scale products scored comparable values of BI with 102.9 and 104.6, as against 95.6 of fresh mango (**Table 6.3a**). The extent of browning with respect to fresh mango was 7.1% and 9.5% for products made in bench and manufacturing scale, respectively. Increase in the BI values could be attributed to non-enzymatic browning of candied mango slices (Chakraborty et al., 2016; Kaushik et al., 2014). The collated processing effects of osmotic treatment and high temperatures during drying could have caused the browning.

6.3.3.3 β-carotene content

The content of β-carotene decreased from 5.5 mg in the fresh sample to 4.8 and 4.7 mg 100 g^{-1} in the candied samples produced in bench and manufacturing scales, respectively (**Table 6.3(a)**). The extent of β-carotene degradation was 12.6 % and 14.4 % for both the manufacturing scales. Exposure of candied mango samples to high temperatures in drying operation could have caused the degradation, as β-carotenes are reported to be temperature sensitive (Lu et al., 2016).

TH-2388_137107006

Table 6.3: Comparison of mango candy (a-b) product characteristics and (c) production time by various processes

(a)

Quality Characteristics	Fresh mango	Candied mango		
		Current study (Bench scale)	Current study (Manufacturing scale)	Commercial product (Manufacturing scale)
Colour properties				
*L**	62.8 ± 0.9^{c}	59.7 ± 1.8^{b}	59.5 ± 0.6^{b}	56.7 ± 0.9^{a}
*a**	14.4 ± 0.5^{b}	13.9 ± 0.4^{a}	13.9 ± 0.4^{a}	20.1 ± 0.6^{c}
*b**	44.7 ± 1.1^{a}	47.7 ± 1^{b}	48.7 ± 0.9^{b}	55.8 ± 1.9^{c}
TCD (*ΔE*)	Reference	4.4 ± 0.1^{a}	5.2 ± 0.2^{b}	12.5 ± 0.2^{c}
Browning Index (BI)	95.6 ± 1.7^{a}	102.9 ± 1.3^{b}	104.6 ± 1.9^{b}	125.1 ± 1.3^{c}
Functional properties				
Vitamin C (mg 100g^{-1})[1]	241.1 ± 1.1^{d}	147.8 ± 0.4^{c}	145.2 ± 0.3^{b}	36.9 ± 0.1^{a}
β-carotene (mg 100g^{-1}) [1]	5.5 ± 0.4^{d}	4.8 ± 0.8^{c}	4.7 ± 0.9^{b}	3.3 ± 0.7^{a}
TPC (mg GAE 100g^{-1}) [1]	67.8 ± 2.3^{c}	54.9 ± 1.4^{b}	52.1 ± 2.9^{b}	22.5 ± 0.9^{a}
Gingerols (mg 100g^{-1})				
After infusion treatment	-	4.4 ± 0.1	4.1 ± 0.1	-
Final product	-	4.1 ± 0.2	3.9 ± 0.2	-

different letters within a row show statistical significance at $p < 0.05$, 1: expressed on a dry weight basis

Table 6.3: Contd.

Quality Characteristics	Fresh mango	Candied mango		
		Current study (Bench scale)	Current study (Manufacturing scale)	Commercial product (Manufacturing scale)
		(b)		
Colour properties				
Appearance (4)	8.3 ± 0.2^{c}	8 ± 0.3^{b}	8.4 ± 0.3^{c}	5.4 ± 0.2^{a}
Mouth Feel (4)	6.8 ± 0.2^{c}	7.3 ± 0.1^{bc}	7.4 ± 0.2^{bc}	6.1 ± 0.3^{a}
After taste (3)	8.1 ± 0.2^{d}	8.2 ± 0.2^{b}	8.2 ± 0.2^{c}	5.9 ± 0.1^{a}
Aroma (4)	6.6 ± 0.3^{c}	7.2 ± 0.1^{a}	7.2 ± 0.2^{b}	5.2 ± 0.1^{a}
Pungency (3)	4.9 ± 0.1^{a}	7.3 ± 0.2^{b}	7.4 ± 0.4^{c}	4.7 ± 0.2^{a}
Sweetness (4)	6.3 ± 0.2^{a}	6.9 ± 0.3^{a}	6.8 ± 0.2^{b}	8.2 ± 0.2^{c}
Sourness (3)	6.6 ± 0.1^{a}	6.9 ± 0.1^{b}	6.9 ± 0.2^{b}	7.2 ± 0.3^{c}
Overall sensory score (OSS)	6.8 ± 0.3^{c}	7.4 ± 0.3^{b}	7.5 ± 0.4^{c}	6.1 ± 0.2^{a}
Physical property				
Shear force ($N\ m^{-1}$)	4.2 ± 0.2^{b}	4.6 ± 0.2^{c}	4.6 ± 0.1^{c}	3.1 ± 0.1^{a}

different letters within a row show statistical significance at $p < 0.05$

TH-2386_136107006

TH-2388_136107006

Table 6.3: Contd.

(c)			
Processing steps	**Time taken (hours)**		
	Current study (Bench scale)[a]	Current study (Manufacturing scale)[b]	Commercial product (Manufacturing scale)[b]
Washing, peeling, de-stoning, cutting, pricking and blanching	0.5	1	1
Gingerols (6+8+10) infusion	5	5 ± 0.1	-
Osmotic treatment	14	16	120 ± 5.5
Drying	4	6 ± 0.1	20 ± 0.5
Sugar layering & packaging	0.5	1 ± 0.1	1 ± 0.1
Transition between processing steps	0.5	1	1
Total	24.5 ± 0.1	30 ± 0.3	143 ± 6.1

a: Bench scale batch size = 0.5 kg with manual operation of 1person, b: commercial scale batch size = 100 kg with manual operation of 8 persons

Guwahati

β-carotene is a natural pigment, responsible for the reddish-yellow color of mango fruit. Hence, β-carotene degradation directly correlates to browning of the samples.

6.3.3.4 Gingerols content

Gingerols content of infused mango slices before the candying operation was found to be 4.4 mg 100 g^{-1} for bench scale and 4.1 mg $100g^{-1}$ for manufacturing scale product. At the end of the candying and drying operation, the gingerols content reduced to 4.1 mg $100g^{-1}$ and 3.9 mg $100g^{-1}$ for bench and manufacturing scales, respectively. This implied that there was a slight gingerols content loss of 0.3 mg $100g^{-1}$ (6.8 %) and 0.27 mg $100g^{-1}$ (6.6 %) for bench and manufacturing scales, respectively, during the candying and drying operation. When fruit matrix infused with water soluble bioactives is candied in hypertonic sugar solutions containing dissolved bioactives, the fruit can still loose the infused bioactives to the surrounding osmotic medium due to osmotic dewatering of fruit.

The results of our study are in accordance with findings of a similar study on anthocyanin infused Amla candy (Adsare et al., 2016), where anthocyanin content of *Amla* slices reduced from 3.15 mg $100g^{-1}$ to 3.05 mg $100g^{-1}$, during candying in sugar syrups containing anthocyanins.

6.3.3.5 Shear strength and sensory scores

Shear strength is calculated to replicate the shearing action of the front incisors to cut food when introduced in the mouth. Calculated average values to cut the samples into two halves were 4.18 N m^{-1} for fresh mango, and 4.6 N m^{-1} for candied mango obtained from bench and manufacturing scales, respectively, as presented in **Table 6.3(b)**. The increase in shearing strength of 9.3 % and 8.9 % for both the samples showed improvement of chewing quality and hence the crunchiness of the product. This is also reflected in the increased scores of the mouthfeel parameter of the overall sensory score values of candied mango produced in the bench and manufacturing scale over the fresh mango scores.

Overall sensory scores (*OSS*) were 7.4 and 7.5 for the product made under bench and manufacturing scales, respectively as against 6.8 for fresh mango samples. Thus, improvement recorded in sensory scores were 7.8 % and 9.5 % higher than the fresh mango scores for both scales, respectively. The collated effects of gingerols infusion and

improved process resulting in better color retention and improved chewing properties, resulted in substantial improvement of individual sensory parameters of product's appearance, mouthfeel, after taste, aroma, pungency sweetness and sourness (**Table 6.3(b)).** This improvement ultimately reflected in higher overall sensory scores of gingerols infused candied mango produced in manufacturing scale with optimized process parameters.

6.4 Comparison with a commercial candied mango product manufactured by existing industrial process and similar published literature

It is often useful to compare the results of process scale-up studies with the product quality and process characteristics of a similar commercial product existing in the market. Such comparisons give invaluable insights on how the results of performed study compare against the existing market trends in candied fruits segment. This ultimately helps in making critical choices towards the commercialization of the product.

A popular commercial candied mango product has been compared with gingerols infused product developed in the current study, produced employing the process optimized in bench and commercial scale facility. The non gingerols infused commercial product was produced employing a commercially practiced process which involved candying treatment for 24 h each in 30-70 ^{o}Brix sugar solutions followed by tray drying for 20 h (as detailed in **Fig. 6.1**). For the sake of comparison, all other process parameters like batch size, slices to solution ratio along with pre and post production steps were kept same.

Detailed quality characteristics comparison is presented in **Table 6.3(a)** and breakup of time taken by individual processing steps for each product has been compared in **Table 6.3(c)**. Among all steps, difference in residence time of mango slices in different osmotic solutions during the candying process as well as in dryers was responsible for the difference in the product quality parameters.

Bench and manufacturing scale products of the current study did not differ significantly (p<0.05) from each other in terms of quality traits. An increase in 2 h residence time each in osmotic solutions and vacuum dryer contributed to this slight deviation between both scales, which was within 5% difference range. However, at manufacturing scale settings, the total process time of 30 h for the gingerols infused product was substantially lower than total process time of 143 h for the commercial product. It was due to significant difference of 104 h in osmotic treatment and 14 h in

TH-2388_136107006

drying processes. The substantially lower residence time of gingerols infused candied mango slices in osmotic solution as well as dryer, resulted in lesser leaching and temperature degradation of parameters associated with nutraceutical qualities of the product. Due to this reason, an improvement of 293.6 %, 131.9 %, and 42.6 % in the retention of vitamin C, TPC, and β-carotene contents, respectively was observed in gingerols infused product over commercial product. In addition, overall sensory scores and shear strength improved by 22.1 % and 46.3 %, respectively. Increased process time of the commercial product also contributed to 140.4 % increase in the total color difference and a 19.5 % increase in the browning index. Hence, 376.7 % reduction in overall process time with a substantial improvement in all the quality characteristics was observed in the product of the current study when compared with a commercially available candied mango product.

In addition to comparison with a commercial candying process as practiced in India, comparison with similar published works on OD/candied mango from different regions of the world is also vital for understanding the deviation in the results obtained in the current study with that of literature. **Table 6.4** compares the effects of process parameters optimized in the study *viz*. bioactive infusion, OD/candying process and drying operation on some critical quality determining factors of different mango varieties reported in literature. Mango is known for its rich contents of natural antioxidants, vitamin C and β-carotene. Hence, the retention of these functional components becomes important while choosing a processing technique. Vitamin C retention of 61.3 % along with 81 % of total phenolics in the current study was highest among all the bench scale studies.

However, β-carotene retention of 87.4 % was found to be much lower in the current study as compared to 95.8 % for Mexican study on Creole variety of mango (Jiménez-Hernández et al., 2016). This is mainly because the infusate chosen by them was pepper oleoresin, which is known for its rich carotenoids contents. This might have contributed to the increased β-carotene contents. Comparison of other results such as water activity and total phenolics contents of present study with Mexican variety showed almost similar trend, except for vitamin C where we observed low retention of 54 %. To the best of our knowledge, no systematic study has been reported involving scale-up of process parameters optimized at bench scale. Hence, substantial improvement in the quality and process time achieved in the current study, shows highly promising commercialization potential of gingerols infused candied mango product.

TH-2388_136107006

Table 6.4: Literature comparison of the current study with various published reports

Sr. No.	Cultivar of mango used	Infusate	Details of optimum process parameters			Final product quality characteristics	Reference
			Infusion	Candying/OD	Drying		
Bench-scale study							
1	*Fazli* (India)	Gingerols	Solution: 0 °Brix + infusate (gingerols conc. 300 mg L^{-1}) Temperature: 27 °C Treatment time: 5 h Fruit/solution ratio: 1/5	30-70 °Brix sucrose solution + infusate (300 mg L^{-1}) Temperature: 27 °C Time: 14 h Fruit/solution ratio: 1/5	Vacuum drier Vacuum: 90 KPa Temperature: 60 °C Time: 2 h	Vitamin C: 61.3 %[a] β-carotene: 87.4 %[a] Total Phenolics: 81 %[a] Gingerols: 4.42 mg $100g^{-1}$[a] Moisture content: 13.5 %[b] a_w = 0.45	Current Study
2	*Creole* (Mexico)	Inulin + pequin-pepper oleoresin	Combined OD + infusion Osmotic solution of 60 wt % (25 % infusate + 45 % sucrose) Temperature: 40 °C Treatment time: 120 min Fruit/solution ratio: 1/30		Oven drier Temperature: 80 °C Time: 24 h	Vitamin C: 54 %[a] β-carotene: 95.8 %[a] Total Phenols: 79.9 %[a] Total oil: 2.22 g $100g^{-1}$[a] a_w = 0.47	(Jiménez-Hernández et al., 2016)
3	*Tommy Atkins* (Sweden)	Calcium ($CaCl_2$)	Combined OD + infusion Sugar solution of 45 °Brix + 1 wt % infusate Temperature: 25 °C Treatment time: 15 h Fruit/solution ratio: 1/10		Hot air drier Air vel.: 0.2 m s^{-1} Temperature: 70 °C Time: 2 h	Vitamin C: 60 %[a] β-carotene: 76.9 %[a] a_w = 0.61 Moisture content: 16 %[b]	(Guiamba et al., 2016)

a: % retention with respect to values of fresh mango on a dry weight basis, b: expressed on a dry weight basis, a_w: water activity

Table 6.4: Contd.

Sr. No.	Cultivar of mango used	Infusate	Details of optimum process parameters			Final product quality characteristics	Reference
			Infusion	Candying/OD	Drying		
Bench-scale study							
4	*Alphonso* (India)	Aloe Vera gel	Combined OD + infusion Sugar solution of 45 °Brix + 5 wt % infusate Temperature: 45 °C Treatment time: 60 min Fruit/solution ratio: 1/20		Vacuum drier Vacuum: 0.0005 KPa Temperature: 45 °C Time: 2 h	Vitamin C: 36 %[a] Total Phenols: 51.5 %[a] Moisture content: 1.5 %[b]	(Chakraborty and Samanta, 2015)
Manufacturing scale study							
1	*Fazli* (India)	Gingerols	Solution: 0 °Brix + infusate (gingerols 300 mg L^{-1}) Temperature: 27 °C Treatment time: 5 h Fruit/solution ratio: 1/5	30-70 °Brix sucrose solution + infusate (300 mg L^{-1}) Temperature: 27 °C Treatment time: 16 h Fruit/solution ratio: 1/5	Vacuum drier Vacuum: 90 KPa Temperature: 60 °C Time: 6 h	Vitamin C: 51 %[a] β-carotene: 85.6 %[a] Total Phenolics: 76.8 %[a] Gingerols: 4.12 mg $100g^{-1}$[a] Moisture content: 13.5 %[b] a_w = 0.45	Current Study

a: % retention with respect to values of fresh mango on a dry weight basis, b: expressed on a dry weight basis, a_w: water activity

6.5 Summary

The collated effects of various process improvement measures undertaken for developing gingerols infused candied mango product resulted in a drastic improvement of the nutritional and functional quality of candied mango slices. Gingerols infusion into mango matrix not only improved the functional quality but flavor synergy of gingerols with mango was also responsible for the enhancement in overall sensory scores. Key results could be summarized as under:

- Optimization of the candying process for maximum sugar and water loss of mango slices resulted in minimum processing loss of natural bioactive compounds present in the mango.
- Process conditions optimized at bench scale were scaled-up 200-fold and augmented with commercial-scale vacuum drying at a commercial scale candy manufacturing facility.
- The manufactured product resulted in 85.6 %, 76.8 %, 60.2 % retention in β-carotene, total phenolics, Vitamin C, respectively, along with minor color difference and significant improvement in the sensory scores over fresh mango.
- A strong agreement in mass transfer properties and all the quality characteristics within bench and manufacturing scale experiments implied excellent scalability and repeatability of the candying process.
- The current study also resulted in significant enhancement of vitamin C, TPC, and β-carotene contents by 233.3 %, 131.8 % and 42.7 %, respectively along with superior color, sensory and textural properties, compared with an existing market product.
- In addition, the drastic reduction of overall process time from 143 h to 30 h was also observed.
- The present study gives a very simple manual process, which can be used to develop a commercial process of other popular fruit/vegetable based candied products, particularly targeted in reducing postharvest losses in developing countries.

TH-2388_136107006

Chapter 7

Storage studies for the developed ginger based food product

Work published at:

Shukla, A., Das, C., & Goud, V. V. (2020). Infusion of gingerols into candied mango enhances shelf-life by inhibiting browning and associated quality parameters during storage. *Food Chemistry, 316*, 126354.

TH-2386_136107006

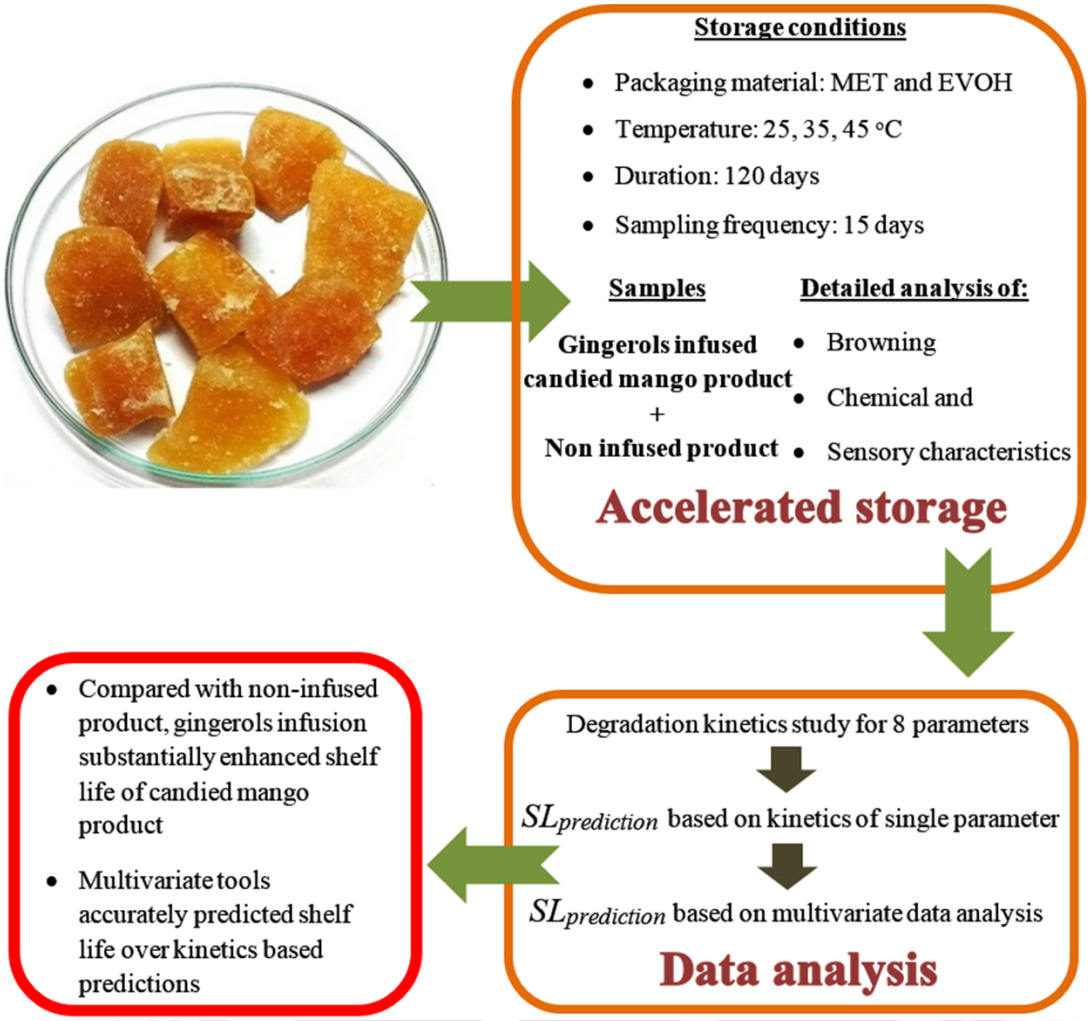
TH-2386_136107006

7.1 Background

Synthetic antioxidants are employed by the industries for enhancement of shelf-life characteristics of commercial food products. A stream of recent literature has demonstrated the carcinogenic effects of synthetic antioxidants on human health. This, coupled with an increasing health awareness of consumers, has directed the focus of research towards finding natural ingredients with green labels for improving shelf-life. Candied fruits are relatively stable products at room temperature but majorly suffer from browning changes during storage. This study presents a green strategy for enhancing the shelf-life of a commercial candied mango product. Infusion of gingerols enriched ginger oleoresin obtained through green solvents substantially inhibited the browning inducing factors during storage. In addition, the unique pungent flavor of gingerols synergistically enhanced the overall sweet-sour flavor spectrum as well as functional qualities of candied mango product. Thus, gingerols enriched oleoresin could be utilized as a cheap and green ingredient for enhancing the shelf-life of other fruit-based industrial products.

7.2 Overview

The unique pungency and high antioxidant potential of gingerols make it an attractive natural flavoring ingredient. The study presents infusion of gingerols enriched oleoresin into candied mango for synergistic enhancement of quality as well as shelf-life characteristics. Gingerols infused candied mango product (GIP), (containing 3.7 mg gingerols $100g^{-1}$) and non-gingerols infused product (control) were packed in multilayer metalized (MET), and ethylene vinyl alcohol (EVOH) based pouches and stored at 25, 35 and 45 ^{o}C for 120 days. Univariate degradation rates of quality parameters showed following order; $k_{\beta\text{-carotene}} > k_{sensory\ (color)} > k_{non\text{-}enzymatic\ browning} > k_{vitamin\ C} > k_{antioxidant\ capacity} > k_{sensory\ (overall)} > k_{total\ phenolics} > k_{gingerols}$, resulting in multiple cutoff criteria and predicted shelf-lives ($SL_{predicted}$). Application of multivariate data analysis tools simplified the kinetic interpretations and hence resulted in accurate predictions. Gingerols infusion retarded the deterioration of all quality parameters and substantially enhanced $SL_{predicted}$ of GIP over control, irrespective of storage conditions.



Graphical abstract of the work presented in the chapter

7.3 Results and discussion

7.3.1 Changes in quality parameters during storage

Critical parameters studied in this work which are responsible for browning of candied mango during storage were: vitamin C, β-carotene, non-enzymatic browning, total phenolic content, antioxidant capacity, sensory score profiles, and gingerols. The variation of studied parameters with time is presented in **Fig. 7.1** and their kinetic parameters in **Table 7.1**. The effects of storage temperature, as well as packaging materials, have visible signs of induced browning on the candied mango samples. This could be due to various factors, namely, oxygen content in the pouch headspace, temperature, chemical composition of product, etc., which have been discussed in details in the following sections.

7.3.1.1 Browning characteristics

The changes in Browning Index (BI) values with respect to time are presented in **Fig. 7.1 (a1 and a2)** and the condition of the products after 120 days of storage is shown in **Fig. 7.2**. The increase in BI values from 0 to 120 days of storage was 29.5 %, 72.4 %, 122.9 % for gingerols infused samples in MET pouches and 42.9 %, 82.9 %, 133.3 % for EVOH based pouches when stored at 25, 35 and 45 ^{o}C, respectively. Similarly, for non-gingerols infused samples (control), the increment was 60.4 %, 99.1 %, 156.6 % and 68.9 %, 107.6 %, 163.2 % for MET and EVOH pouches at 25, 35 and 45 ^{o}C, respectively.

Thus, gingerols infusion had the most pronounced effect on non-enzymatic browning in candied mango samples, even at the lowest temperature of 25 ^{o}C among all the factors. Non-enzymatic reactions are reported to be responsible for the formation of brown pigments during the storage of food products (Wibowo et al., 2018). These reactions are reported to occur through various pathways, for example, the degradation of sugars, Maillard reactions, etc.

Amongst them, the most critical reaction mechanism is the degradation of vitamin C into various products. In addition, depletion of β-carotene is also responsible for browning (Syamila et al., 2019) of the candied mango product. The detailed discussions about these factors have been explained in the subsequent sections.

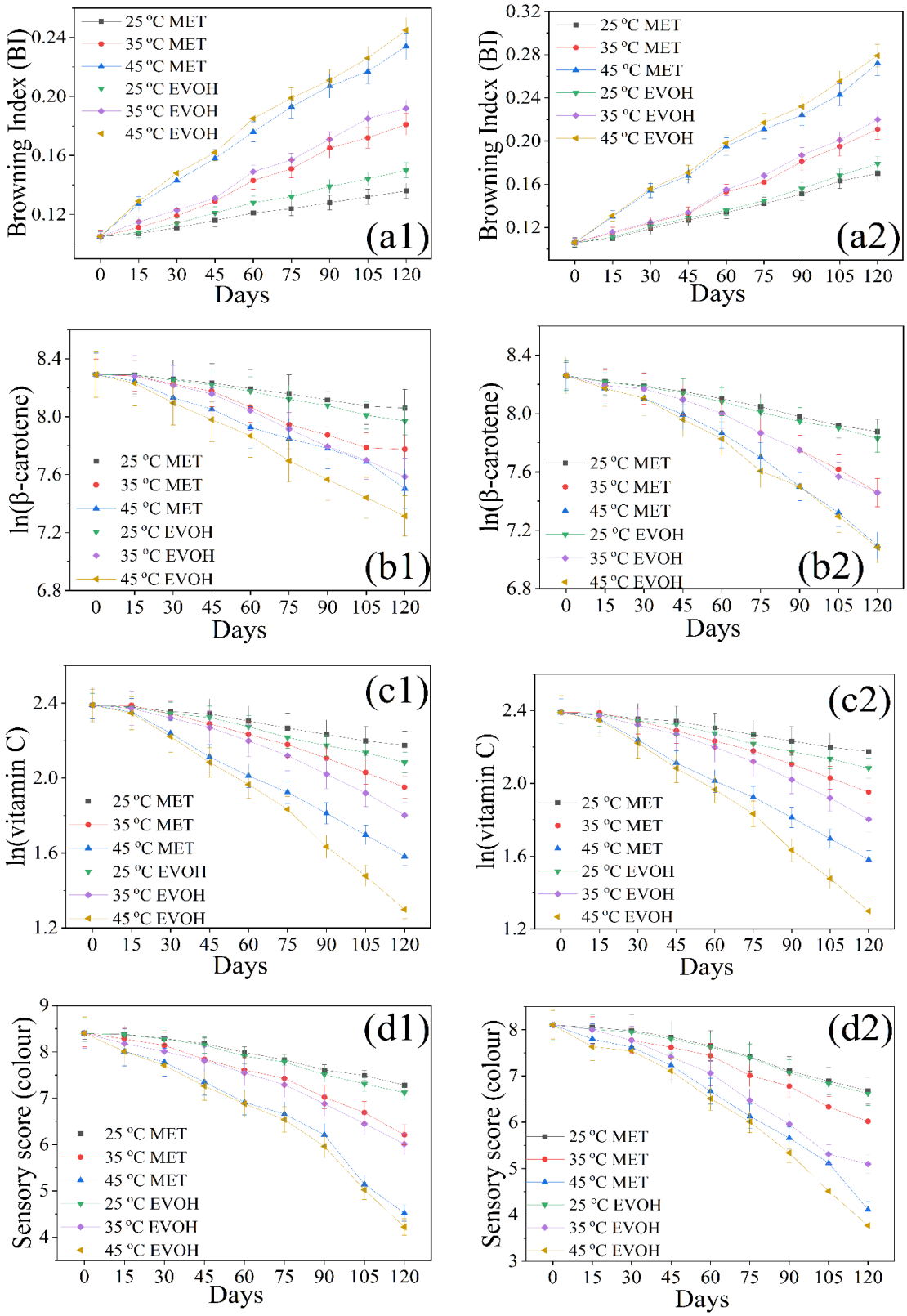


Fig. 7.1: Variation of (a) browning, (b) β-carotene, (c) vitamin C and (d) sensory scores of colour at different storage conditions of temperatures (25 ºC, 35 ºC and 45 ºC) and packaging materials (MET and EVOH) for gingerols incorporated candied mango samples (index:1) and non gingerols incorporated samples (index:2)

TH-2388_136107006

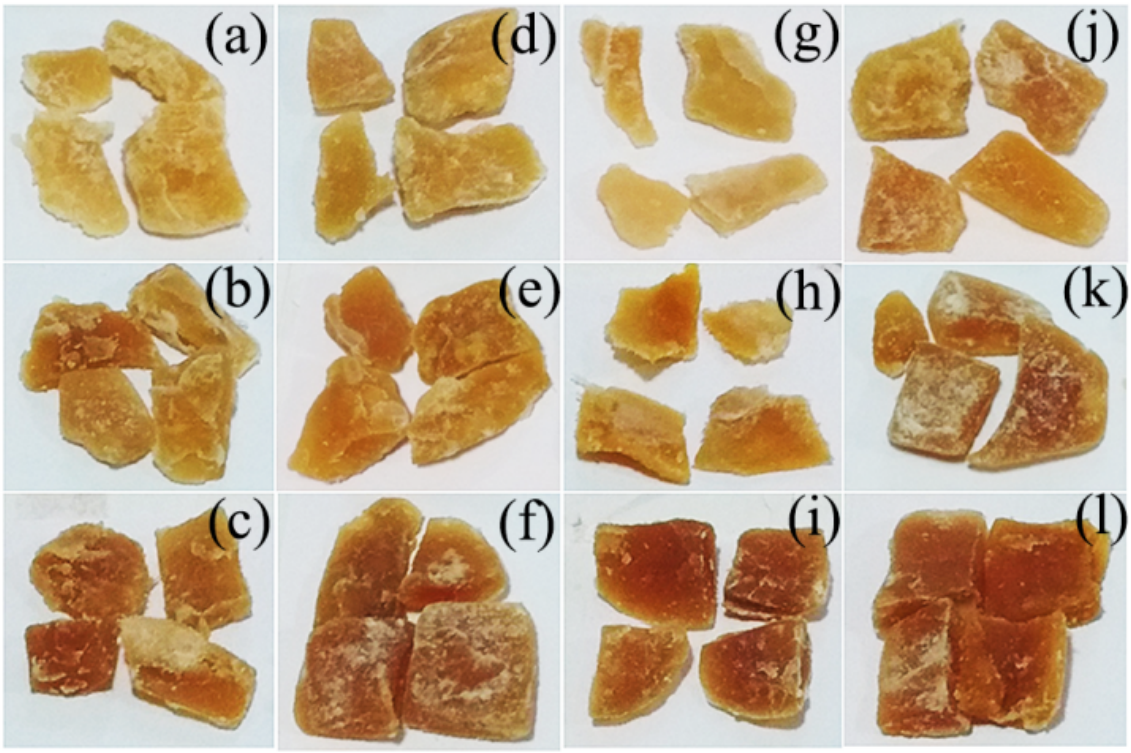


Fig. 7.2: Condition of samples at the end of 120 days when stored at 25 °C (first row, a-j), 35 °C (second row, b-k), and 45 °C (third row, c-l), first column (a, b and c) represents gingerols incorporated samples in MET packaging, second column (d, e and f) in EVOH, similarly control samples in MET is shown by third column (g, h and i) and control samples in EVOH by fourth column (j, k and l)

7.3.1.2 β-carotene, total phenolics, and antioxidant capacity

β-carotene is the major pigment found in mango which is responsible for its yellowish-orange colour. The change in β-carotene's content is critical for studying color degradation of candied mango samples during storage. Initial (day 0) values of β-carotene were found to be 3985.7 µg and 3866.1 µg per 100 g of sample for gingerols infused and non-infused (control) products, respectively. At the end of 120 days, β-carotene contents degraded by 20.6 %, 40.3 %, 54.5 % for samples of MET pouches and 27.4 %, 50.6 %, 62.3 % for EVOH when stored at 25, 35 and 45 °C, respectively. Similarly, for control samples, the degradation was 31.9 %, 45.2 %, 68.8 % and 35 %,

55.7 %, 71.2 % for MET and EVOH pouches at 25, 35 and 45 ^{o}C, respectively, as presented in **Fig. 7.1(b1 and b2)**.

The change in water activities for both kinds of candied mango samples (control and gingerols infused) in all conditions of storage and temperatures were within a range of 0.4-0.45. At such low water activities, β-carotene has been reported to remain stable under long term storage at low temperatures, for products naturally rich in β-carotene like dehydrated carrots (Caparino et al., 2017). The candied mango samples stored at 45 ^{o}C showed highest β-carotene degradation compared to 25 ^{o}C, irrespective of their antioxidant potential and packaging material. Hence, the extent of β-carotene degradation was directly dependent on the magnitude of storage temperature.

The oxygen content of the storage environment was also found to have a direct effect on the deterioration of β-carotene. The samples kept in pouches with MET configuration were hence better preserved in β-carotene contents, as compared to those in EVOH pouches. The presence of the metalized layer of aluminium in MET pouches was responsible for low oxygen permeability from the ambient environment as a contrast to non-metal EVOH based structure. These observations suggest the auto-oxidative and thermal degradation mechanism of β-carotene. The possible reaction pathways include isomerization of all-*trans*-β-carotene into *cis*-isomers along with the opening of the β-ionone ring under the effect of oxygen and temperature as well as the formation of epoxides under the exposure of free radicals (Syamila et al., 2019), as presented in **Fig 7.3**.

Infusion of gingerols improved the antioxidant potential of candied mango samples by 15.5 % in antioxidant capacity and 15.6 % in total phenolic contents, respectively, as compared to the control samples (**Fig 7.4**). This resulted in arresting the free radicals formed due to the effects of temperature, oxygen, and other biochemical reactions in the gingerols infused product. As a result, the degradation of β-carotene contents in gingerols infused product was 10.3 %, 14.9 %, 14.3 % less for MET and 7.6 %, 5 %, 6.9 % less for EVOH based pouches, as compared to the non-infused products (control), at the end of 120 days when stored at 25, 35 and 45 ^{o}C, respectively due to lower epoxide formation.

7.3.1.3 Vitamin C and gingerols

Degradation of ascorbic acid (vitamin C) has been reported to be one of the major factors responsible for inducing browning, ultimately leading to the deterioration of color

TH-2388_136107006

and hence shelf life of mango based products (Caparino et al., 2017). Average estimated values of vitamin C were 140.9 mg $100g^{-1}$ and 138.3 mg $100g^{-1}$ for gingerols infused and control samples, respectively at day zero. After 120 days of storage, the extent of vitamin C degradation followed a similar trend to that of β-carotene's values, as shown in **Fig. 7.1 (c1 and c2)**. For infused samples stored in MET pouches, vitamin C degraded by 19.3 %, 35.5 %, 55.4 %, and for EVOH, 26.4 %, 44.5 %, 66.5 %, at 25, 35 and 45 ^{o}C, respectively, with respect to day 0 values.

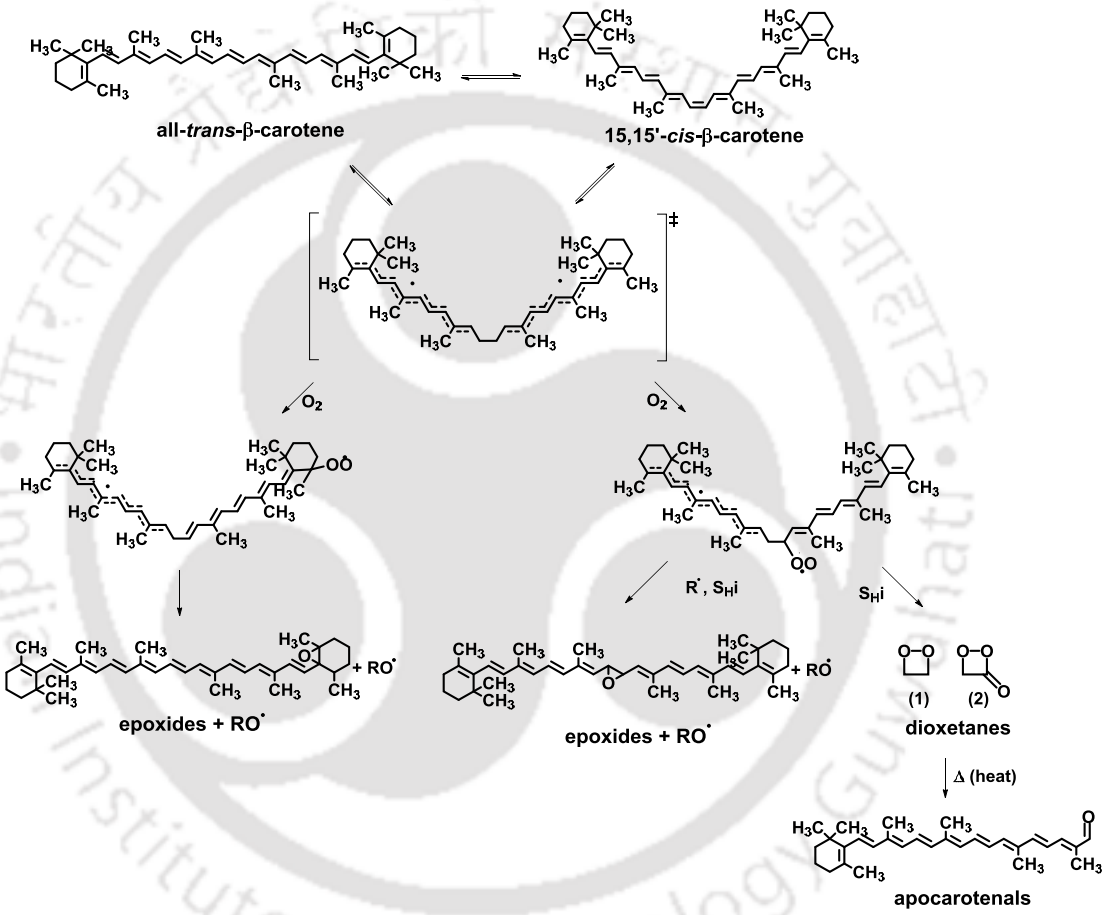


Fig. 7.3: β-carotene degradation mechanism under oxygen exposure and radical attack. [Adapted from Mordi (1993) and Pénicaud et al. (2011)]

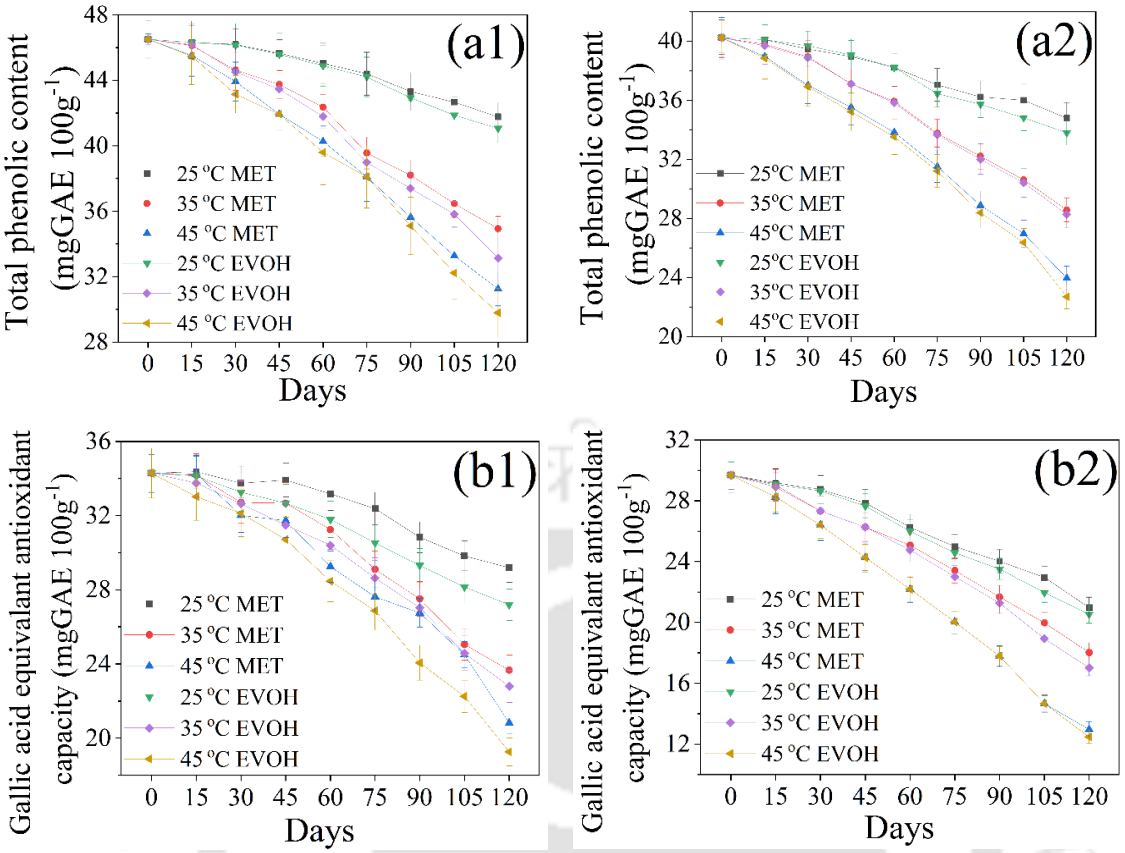


Fig. 7.4: Variation of (a) total phenolic content, and (b) antioxidant capacity at different storage conditions of temperatures (25 °C, 35 °C and 45 °C) and packaging materials (MET and EVOH) for gingerols incorporated candied mango samples (index:1) and non gingerols incorporated samples (index:2)

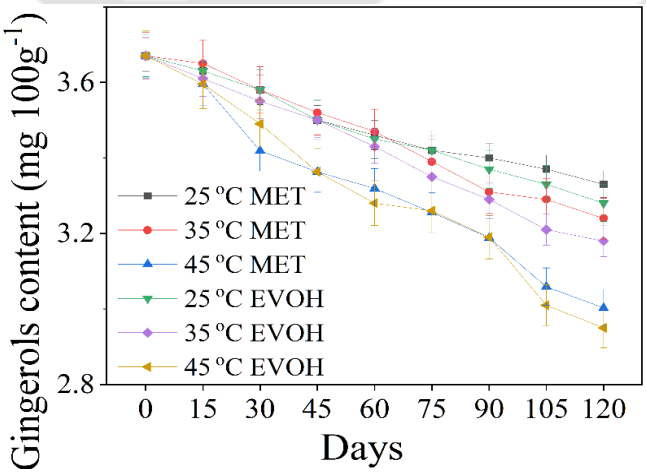


Fig. 7.5: Retention of gingerols content at different storage conditions of temperatures (25 °C, 35 °C and 45 °C) and packaging materials (MET and EVOH) for gingerols incorporated candied mango samples

TH-2388_13610706

For control samples, the degradation was 38.1 %, 50.8 %, 67 % and 40.7 %, 52.1 %, 67.4 % for MET and EVOH pouches at 25, 35 and 45 ^{o}C, respectively. The underlying degradation mechanism of ascorbic acid is its conversion into dehydro-ascorbic acid, which ultimately hydrolyses to 2,3-diketogluconic acid (Wibowo et al., 2015). Slow degradation observed for samples of MET pouches due to lower oxygen transmission rates may have also contributed towards preserving β-carotene contents when compared to samples stored in EVOH based pouches. The degradation of gingerols in gingerols infused samples, as presented in **Fig. 7.5** also followed a similar trend.

7.3.1.4 Sensory profiles

The average initial scores (day 0) for gingerols infused candied mango samples were: 8.4, 6.45, 7.15, 7.21, 7.45, 7.77, 6.85 and 7.37 for color, mouthfeel, after-taste, aroma, pungency, off-flavor, sourness, and overall sensory scores, whereas for control product the scores were: 8.1, 6.25, 7.15, 7.1, 6.5, 7.75, 6.9 and 7.15, respectively. Thus, in addition to increasing the antioxidant potential of candied mango samples, the infusion of gingerols also improved the sensory scores. The characteristic pungency of the ginger actives synergistically worked with the rich flavor of mango in enhancing the overall flavor spectrum of the final product. This is reflected in scores of all the individual sensory parameters chosen for the sensory analysis.

Amongst all the 7 individual sensory attributes, the extent of decrease in sensory scores of color with respect to time during storage, as shown in **Fig. 7.1 (d1 and d2)**, was found to be the most significant. The sensory scores of color for infused samples stored in MET pouches, decreased by 13.3 %, 26 %, 46.2 %, and for EVOH, 15.2 %, 28.5 %, 49.8 %, at 25, 35 and 45 ^{o}C, respectively, with respect to initial scores. For control samples, the sensory color scores decrease by 17.5 %, 25.7 %, 49.3 % and 18.3 %, 37 %, 53.5 % for MET and EVOH pouches at 25, 35 and 45 ^{o}C, respectively. Clearly, higher storage temperature and oxygen permeability of packaging material resulted in rapid browning changes in the candied mango samples, which ultimately reduced the acceptability of the product.

The results are also supported by the changes in non-enzymatic browning, β-carotene, vitamin C, and antioxidant potential of the product. As shown in **Fig. 7.6 (a1 and a2)**, the changes in overall sensory scores during storage were also found to follow the same trend irrespective of storage conditions and duration.

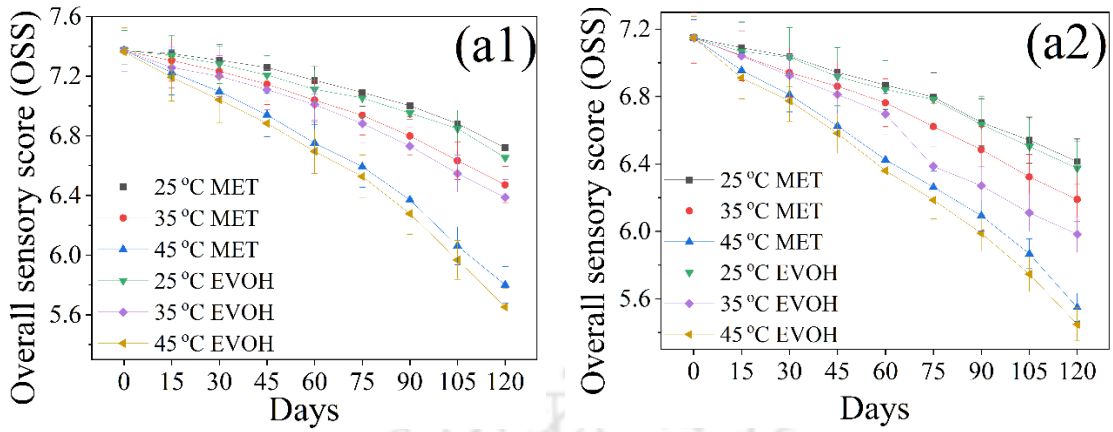


Fig. 7.6: Variation of overall sensory scores (OSS) at different storage conditions of temperatures (25 °C, 35 °C and 45 °C) and packaging materials (MET and EVOH) for gingerols incorporated candied mango samples (a1) and non gingerols incorporated samples (a2)

7.3.2 Univariate degradation kinetics of quality parameters and shelf life markers

The kinetic behaviour of most rapidly changing quality parameter during storage is critical for shelf-life determination of a product. **Table 7.1** presents the kinetic parameters of the estimated quality parameters during the storage of candied mango product. The kinetic rate of change for total phenolic content, antioxidant capacity, non-enzymatic browning, sensory scores of color, overall sensory scores and gingerols followed zero-order reaction kinetics whereas, vitamin C and β-carotene followed first-order kinetics.

Phenolic compounds are known to delay the browning changes in fruits during storage. In addition, the storage behaviour of non-enzymatic browning along with antioxidant capacity is not well known for the case of mango based products. The calculated E_a for total phenolics were in the range of 45-35 kJ mol^{-1}, which was higher than 33-23 kJ mol^{-1} of antioxidant capacity for all conditions of storage and treatment, suggesting greater thermal sensitivity of phenolic compounds in mango during storage than antioxidant compounds. The observed zero-order kinetics for change in total phenolic content and antioxidant capacity during storage is in agreement with antioxidant capacity for pineapple puree (Chakraborty et al., 2016).

Non-enzymatic browning, however, followed a reverse trend of increase as against the decreasing trend of functional parameters.

TH-2388_136157006

Table 7.1: Degradation rates and shelf life data on candied mango samples at different storage conditions based on conventional zero and first-order kinetics of individual quality parameters

Shelf life variables	Gingerols infused in MET			Gingerols infused in EVOH			Non-infused in MET			Non-infused in EVOH		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
Kinetics order		Zero			Zero			Zero			Zero	
$k_{Total\ Phenolics\ Content}$ × 10^{-2} (day^{-1})	4.1 ± 0.03^{a}	10.26 ± 0.11^{b}	13.13 ± 0.13^{c}	4.77 ± 0.02^{a}	11.47 ± 0.14^{bc}	14.06 ± 0.17^{c}	4.72 ± 0.04^{a}	10.11 ± 0.08^{b}	13.48 ± 0.1^{c}	5.8 ± 0.04^{a}	10.32 ± 0.17^{b}	14.29 ± 0.21^{c}
R^2 (adjusted)	0.96	0.98	0.99	0.94	0.99	0.99	0.98	0.98	0.99	0.96	0.98	0.98
E_a (kJ mol^{-1})		45.12 ± 1.12^{d}			40.63 ± 1.49^{c}			37.75 ± 0.97^{b}			34.84 ± 1.37^{a}	
R^2 (adjusted)		0.91			0.90			0.95			0.98	
Shelf life (days)	567	227	177	487	203	165	426	199	149	347	195	141
Kinetics order		Zero			Zero			Zero			Zero	
$k_{Antioxidant\ capacity}$ × 10^{-2} (day^{-1})	4.6 ± 0.02^{a}	9.31 ± 0.16^{c}	10.85 ± 0.16^{cd}	6.26 ± 0.05^{ab}	9.72 ± 0.21^{c}	12.48 ± 0.22^{de}	7.3 ± 0.05^{b}	9.77 ± 0.07^{c}	14.34 ± 0.28^{e}	7.92 ± 0.06^{b}	10.65 ± 0.28^{cd}	14.57 ± 0.21^{e}
R^2 (adjusted)	0.91	0.95	0.96	0.98	0.97	0.98	0.97	0.99	0.99	0.98	0.98	0.99
E_a (kJ mol^{-1})		33.29 ± 1.05^{c}			28.64 ± 0.67^{b}			25.97 ± 0.38^{ab}			23.29 ± 0.41^{a}	
R^2 (adjusted)		0.89			0.98			0.99			0.90	
Shelf life (days)	373	184	158	274	176	137	203	152	103	187	139	102
Kinetics order		First			First			First			First	
$k_{Vitamin\ C}$ × 10^{-3} (day^{-1})	2.22 ± 0.12^{a}	3.81 ± 0.14^{ab}	6.93 ± 0.13^{c}	2.51 ± 0.16^{a}	4.22 ± 0.17^{b}	8.52 ± 0.24^{cd}	4.3 ± 0.19^{b}	5.7 ± 0.22^{bc}	9.2 ± 0.31^{d}	4.2 ± 0.16^{b}	5.9 ± 0.24^{bc}	9.2 ± 0.41^{d}
R^2 (adjusted)	0.98	0.97	0.99	0.98	0.96	0.98	0.98	0.98	0.99	0.98	0.97	0.99
E_a (kJ mol^{-1})		49.73 ± 1.69^{d}			47.07 ± 1.2^{c}			32.04 ± 1.51^{b}			30.17 ± 1.17^{a}	
R^2 (adjusted)		0.99			0.99			0.99			0.99	
Shelf life (days)	362	186	100	288	153	85	178	116	77	169	113	77

different letters denote significant statistical difference ($p < 0.05$) among a row for a fixed parameter

TH-2388_137107006

Table 7.1: Contd.

Shelf life variables	Gingerols infused in MET			Gingerols infused in EVOH			Non-infused in MET			Non-infused in EVOH		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
Kinetics order	First			First			First			First		
$k_{\beta\text{-carotene}} \times 10^{-2}$ (day^{-1})	0.21 ± 0.01^{a}	0.5 ± 0.02^{bc}	0.64 ± 0.02^{c}	0.28 ± 0.01^{ab}	0.63 ± 0.02^{c}	0.85 ± 0.03^{cd}	0.33 ± 0.01^{b}	0.67 ± 0.03^{c}	0.97 ± 0.04^{d}	0.36 ± 0.02^{b}	0.68 ± 0.02^{c}	0.98 ± 0.04^{d}
R^2 (adjusted)	0.98	0.97	0.99	0.97	0.97	0.99	0.99	0.96	0.97	0.99	0.96	0.98
E_a (kJ mol^{-1})	43.19 ± 1.26^{c}			41.67 ± 1.46^{b}			41.68 ± 1.5^{b}			38.7 ± 1.25^{a}		
R^2 (adjusted)	0.98			0.94			0.98			0.98		
Shelf life (days)	300	168	98	219	125	74	200	114	68	185	110	68
Kinetics order	Zero			Zero			Zero			Zero		
$k_{\text{non-enzymatic browning}} \times 10^{-3}$ (day^{-1})	0.31 ± 0.01^{a}	0.7 ± 0.02^{c}	1.1 ± 0.03^{de}	0.41 ± 0.02^{ab}	0.8 ± 0.03^{cd}	1.12 ± 0.04^{de}	0.51 ± 0.02^{b}	0.9 ± 0.04^{d}	1.31 ± 0.06^{ef}	0.63 ± 0.02^{c}	1.1 ± 0.06^{de}	1.41 ± 0.06^{f}
R^2 (adjusted)	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
E_a (kJ mol^{-1})	50.22 ± 2.18^{d}			39.13 ± 1.51^{c}			36.9 ± 1.87^{b}			30.17 ± 1.94^{a}		
R^2 (adjusted)	0.98			0.96			0.99			0.99		
Shelf life (days)	350	141	83	263	119	79	212	112	82	169	113	77
Kinetics order	Zero			Zero								
$k_{\text{gingerols}} \times 10^{-2}$ (day^{-1})	0.29 ± 0.01^{a}	0.39 ± 0.01^{b}	0.54 ± 0.01^{cd}	0.33 ± 0.01^{ab}	0.43 ± 0.02^{c}	0.59 ± 0.02^{d}						
R^2 (adjusted)	0.98	0.99	0.98	0.99	0.99	0.98						
E_a (kJ mol^{-1})	23.94 ± 0.88^{b}			22.36 ± 0.97^{a}								
R^2 (adjusted)	0.99			0.99								
Shelf life (days)	633	460	323	556	417	296						

different letters denote significant statistical difference ($p < 0.05$) among a row for a fixed parameter

Table 7.1: Contd.

Shelf life variables	Gingerols infused in MET			Gingerols infused in EVOH			Non-infused in MET			Non-infused in EVOH		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
Kinetics order		Zero			Zero			Zero			Zero	
$k_{sensory\ (colour)} \times 10^{-2}$	0.98 ±	1.8 ±	3.11 ±	1.13 ±	1.95 ±	3.22 ±	1.26 ±	1.77 ±	3.22 ±	1.29 ±	2.74 ±	3.58 ±
(day^{-1})	0.03^{a}	0.05^{b}	0.11^{c}	0.09^{a}	0.08^{b}	0.12^{c}	0.09^{a}	0.08^{b}	0.12^{c}	0.06^{a}	0.08^{bc}	0.15^{c}
R^2 (adjusted)	0.97	0.97	0.96	0.97	0.97	0.96	0.96	0.97	0.97	0.96	0.97	0.97
E_a (kJ mol^{-1})		44.52 ± 1.58^{c}			40.36 ± 1.69^{b}			39.53 ± 1.53^{b}			36.04 ± 1.26^{a}	
R^2 (adjusted)		0.99			0.98			0.97			0.94	
Shelf life (days)	347	188	106	301	173	102	246	175	96	240	113	87
Kinetics order		Zero			Zero			Zero			Zero	
$k_{sensory\ (overall\ score)} \times 10^{-2}$	0.53 ±	0.74 ±	1.29 ±	0.54 ±	0.8 ±	1.38 ±	0.61 ±	0.79 ±	1.27 ±	0.64 ±	1.02 ±	1.36 ±
(day^{-1})	0.02^{a}	0.03^{b}	0.06^{c}	0.02^{a}	0.02^{b}	0.04^{d}	0.01^{ab}	0.02^{b}	0.05^{c}	0.02^{ab}	0.01^{bc}	0.04^{d}
R^2 (adjusted)	0.95	0.97	0.98	0.98	0.97	0.97	0.98	0.99	0.99	0.98	0.98	0.99
E_a (kJ mol^{-1})		36.08 ± 1.44^{c}			34.18 ± 1.52^{b}			29.11 ± 0.83^{ab}			28.16 ± 0.78^{a}	
R^2 (adjusted)		0.97			0.98			0.97			0.99	
Shelf life (days)	448	317	179	439	292	165	352	272	169	336	211	158

different letters denote significant statistical difference ($p < 0.05$) among a row for a fixed parameter

The zero-order reaction kinetics for non-enzymatic browning changes in candied mango during storage is in agreement with the reported non-enzymatic browning behaviour of vinegar-soybean solution (Chen et al., 2019). The degradation of vitamin C and β-carotene during storage has been widely reported to follow first-order kinetics for a range of mango based products like dehydrated mango powder (Caparino et al., 2017), mango puree (Vásquez-Caicedo et al., 2007) and mango juice (Wibowo et al., 2015).

In general, the kinetic rates for all the quality parameters followed the following order: $k_{\beta\text{-carotene}} > k_{sensory(color)} > k_{non\text{-}enzymatic\ browning} > k_{vitamin\ C} > k_{antioxidant\ capacity} > k_{sensory(overall)} > k_{total\ phenolics} > k_{gingerols}$. Calculated Arrhenius activation energies followed the order of $E_{a\ (gingerols\ infused\ product\ stored\ in\ MET)} > E_{a\ (gingerols\ infused\ product\ stored\ in\ EVOH)} > E_{a\ (control\ product\ stored\ in\ MET)} > E_{a\ (control\ product\ stored\ in\ EVOH)}$. This implied gingerols infused products were more stable than a non-infused product. Moreover, the MET based pouches prevented the deterioration of quality parameters for a longer duration over EVOH based pouches. Clearly, β-carotene was found to be the most limiting factor for all the samples, irrespective of storage conditions, and gingerols infusion. Considering 50 % retention of β-carotene as cut-off criteria, the maximum predicted shelf-life was 300 days for gingerols infused product when stored in MET based pouch at 25 °C. On the other hand, control product when stored in EVOH based pouch at 25 °C, had a shelf-life of 185 days.

7.3.3 Multivariate data analysis

The multivariate data analysis tool, principal component analysis (PCA), was employed to analyse the entire shelf-life experimental data simultaneously. The bi-plot presents the relative spread of quality parameters and their storage data points against the extracted principal components (PCs) for gingerols infused and control samples stored in both types of pouches (MET and EVOH), each for 25, 35 and 45 °C in **Fig. 7.7 (a-c)**, respectively.

For 25 °C, 97.76 % and 1.18 % variation of entire data was explained by the new variables, PC1, and PC2, respectively. Similarly, 98.94 % and 0.59 % variation of the entire data of 35 °C as well as 98.17 % and 1.02 % variation of the entire storage data of 45 °C were explained by PC1 and PC2. In addition, the eigen values for PC1 were 7.82, 7.91, and 7.85 for 25, 35, and 45 °C, respectively (data not shown), which implied that maximum variation of the entire storage data was represented by PC1 alone. The bi-plots presented in **Fig. 7.7** clearly show that non-enzymatic browning falls in the left quadrant

TH-2388_136107006

(negative side of PC1), for all temperatures as against all other quality attributes, which were found in the positive side of PC1.

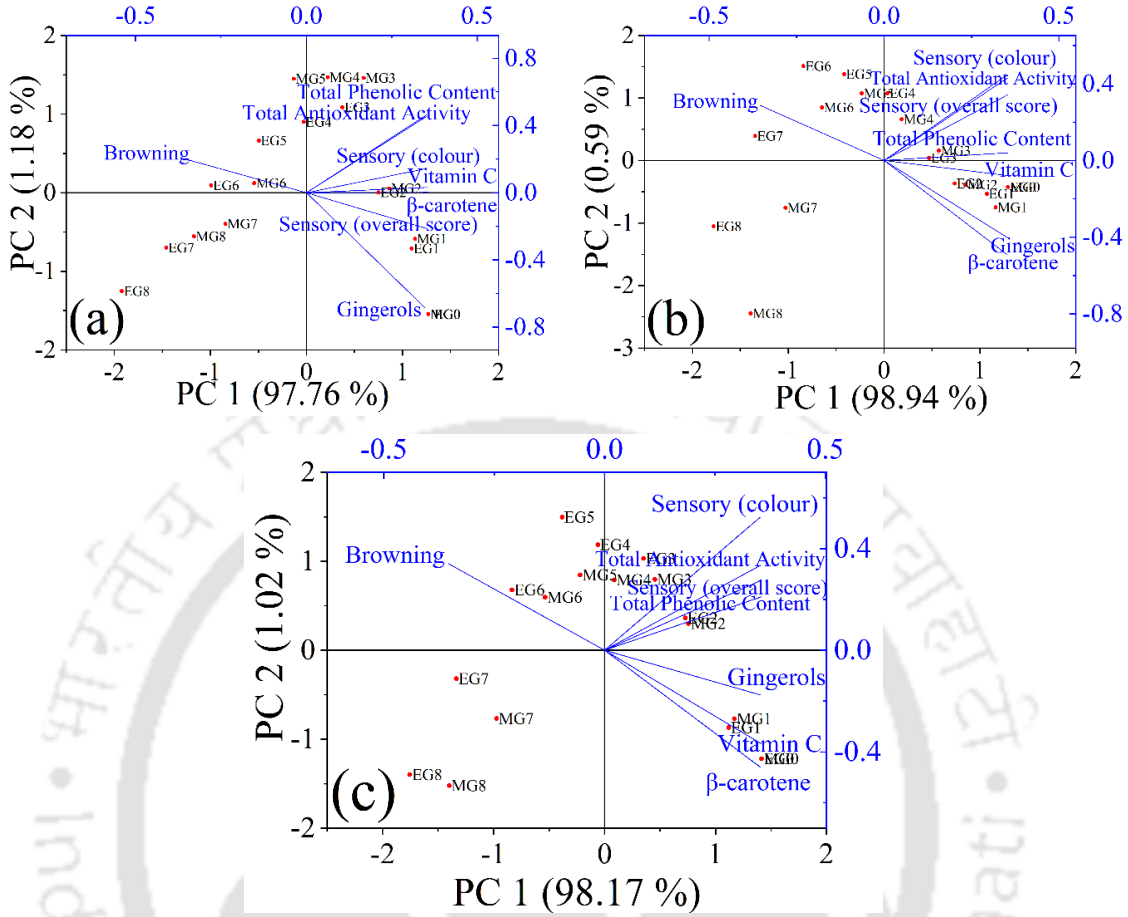


Fig. 7.7: Loading and scores biplots of extracted principle components for different quality parameters of candied mango stored at (a) 25 °C, (b) 35 °C, and (c) 45 °C. Labels 0-8 represent data points at 0-120 days respectively, M and E represent MET and EVOH packaging materials, respectively; G and C stand for gingerols incorporated product and control (non gingerols incorporated product)

This establishes the inverse relationship between non-enzymatic browning and the remaining parameters for all conditions of storage. The data points (presented by red dots in **Fig. 7.7**) corresponding to initial days are observed in the positive side of PC1 along with all the parameters other than non-enzymatic browning, implying higher values of those quality parameters during the initial days of storage.

The degradative changes were more pronounced in the data points of later stages, thus shifting them in the negative side of PC1. This clearly implies that effects of temperature and environment of packaging material showed a negative effect on one set

TH-2388_126107006

Table 7.2: Pearson's correlation coefficients within various measured quality parameters of candied mango samples under different storage conditions

Parameters	Total Phenolic Content	Antioxidant Activity	β-carotene	Non-enzymatic browning	Vitamin C	Sensory (overall score)	Sensory (color)
Antioxidant Activity	0.98						
β-carotene	0.79	0.89					
Non-enzymatic browning	-0.76	-0.87	-0.98				
Vitamin C	0.56	0.71	0.94	-0.95			
Sensory (overall score)	0.95	0.88	0.59	-0.57	0.32		
Sensory (colour)	0.80	0.90	0.99	-0.97	0.92	0.60	
Gingerols	0.93	0.92	0.97	-0.97	0.97	0.97	0.97

Coefficients significant at $p < 0.005$

of quality attributes namely, TPC, AC, vitamin C, β-carotene, sensory (overall scores), sensory (color), as well as gingerols, in contrast to browning which increased with time.

This observation has also been supported by Pearson's correlation coefficients (r) among all quality attributes, as presented in **Table 7.2**. The non-enzymatic browning inversely correlated ($r \geq -0.95$) very strongly with vitamin C, β-carotene, sensory scores of color, and gingerols content. This also supports our proposed degradation mechanism of vitamin C and β-carotene producing brown pigment compounds. In contrary, the gingerols content was very strongly correlated ($r \geq 0.92$) with all the studied quality attributes, mainly because of the synergistic effect of gingerols on retarding the changes in sensory scores and parameters which are critically responsible for browning of candied mango samples during storage.

7.3.3.1 Shelf-life prediction based on multivariate data analysis

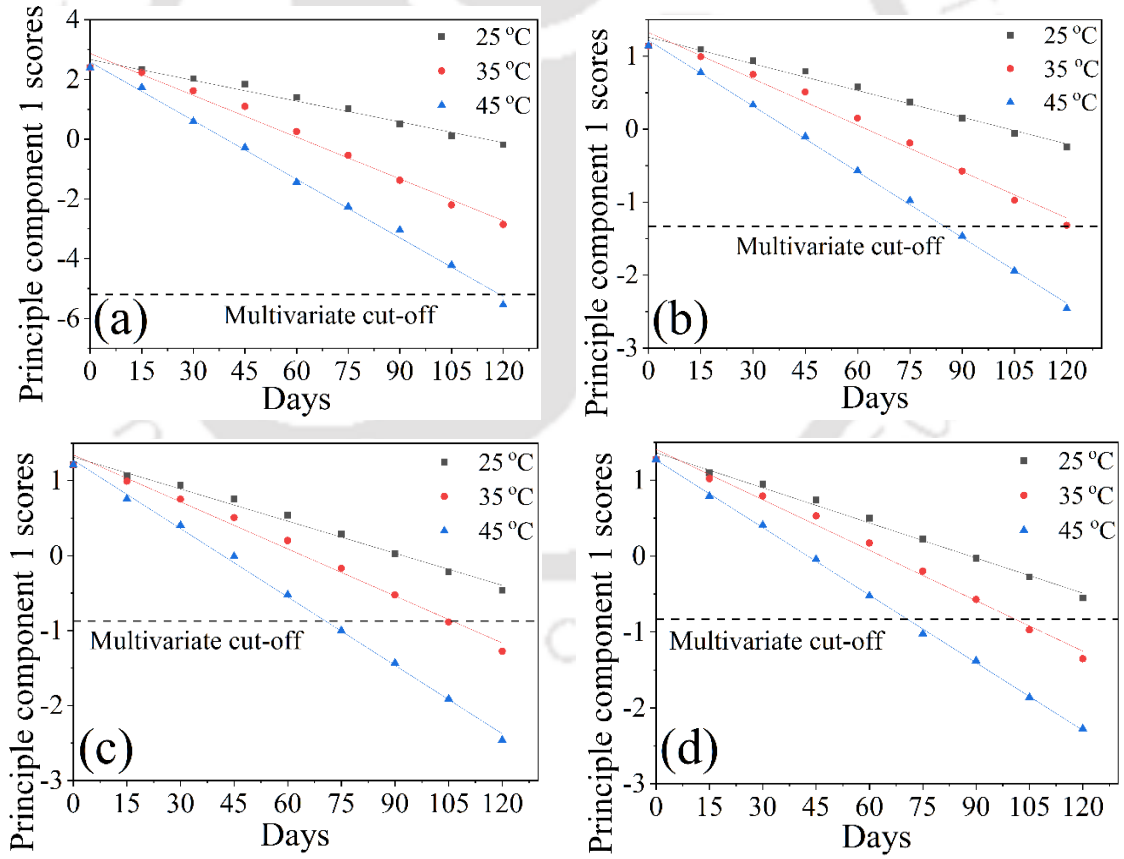


Fig. 7.8: Variation of PC1 scores for gingerols incorporated samples stored in (a) MET, (b) EVOH, (c) non gingerols samples in MET, and (d) non gingerols samples in EVOH based packaging materials at different temperatures (25 °C, 35 °C and 45 °C). Dotted lines represent multivariate cut-off criteria

A holistic estimation of shelf-life not only considers the variations of single most abruptly changing parameter, but also considers the effects of spoilage mechanisms of other parameters along with their possible interactive effects (Sehwag et al., 2018). Thus, the PC1s extracted from the storage data matrix of all quality parameters for all conditions of storage and treatments represented ≥ 97 % variation of the entire data and were used for estimation of kinetic parameters and prediction of shelf lives.

Extracted PCs plotted against time were found to fit zero-order kinetics for all cases and showed more accurate fitting (with R^2 values lying between 0.97 to 0.99, **Fig. 7.8**) compared to the univariate kinetic fitting of individual quality parameters (where R^2 values were between 0.91 to 0.99, **Table 7.1**). Owing to better-fitting, the new rate constants $k_{multivariate}$, and the new temperature acceleration factor ($\alpha^T_{multivariate}$), represented the entire shelf-life data better (**Table 7.3**) compared with single univariate parameter based kinetics parameters (**Table 7.1**). Based on new cut-off criteria calculated from these factors, the predicted shelf-lives calculated were 338, 173, 119 days for gingerols infused product stored in MET pouch at 25, 35 and 45 ^oC and 212, 125, 85 days for EVOH pouch, respectively. Whereas, for control product, estimated shelf-lives were 155, 107, 71 days for MET pouch at 25, 35 and 45 ^oC and 144, 104, 73 days for EVOH pouch, respectively (**Table 7.3**). These values were significantly different from the univariate kinetics based estimated shelf lives.

Hence, in order to further prove our hypothesis and understand the preciseness of predictions, these values were compared with the actual shelf life estimated for non-gingerols infused product in EVOH packaging at normal temperature (25 ^oC). The actual shelf life for the product was observed to be 142 days, which was quite close to the multivariate based predicted shelf life of 144 days for same storage conditions. There was an over prediction of only 2 days (1.4 %) compared to the actual value. In contrast, the predicted shelf-life based on univariate kinetics considering β-carotene as a single and most abruptly degrading parameter was 185 days for same storage conditions. The extent of over prediction was 41 days (28.5 %), which was substantially higher than the actual shelf-life. This establishes the preciseness of multivariate data analysis based approach as against the conventional kinetics based approach. Hence, based on the predictions of multivariate analysis, it can be concluded that gingerols infusion can drastically improve the shelf life of candied mango product from 155 days to 338 days at normal temperature conditions (25 ^oC), when stored in metalized based packaging material.

TH-2388_13110706

TH-2386_136107006

Table 7.3: Degradation rates and shelf life data on candied mango samples at different storage conditions based on multivariate parametric analysis

Sample details	Storage temperature (°C)	Kinetic order	$k_{multivariate}$ × 10^{-2} (day^{-1})	R^2 (adjusted)	Shelf life (days)	E_a (kJ mol^{-1})	R^2 (adjusted)	Temperature acceleration factor ($\alpha_{multivariate}$)
Gingerols infused in MET	25	Zero	2.32 ± 0.08^a	0.98	338	40.08 ± 1.29^d	0.97	1
	35		4.66 ± 0.13^b	0.98	173			2.0
	45		6.54 ± 0.21^c	0.99	119			2.8
Gingerols infused in EVOH	25	Zero	1.22 ± 0.03^a	0.99	212	34.75 ± 1.34^c	0.99	1
	35		2.12 ± 0.8^b	0.99	125			1.7
	45		3.0 ± 0.07^c	0.99	85			2.5
Non-infused in MET	25	Zero	1.43 ± 0.06^a	0.99	155	29.06 ± 0.92^b	0.99	1
	35		2.09 ± 0.09^b	0.99	107			1.5
	45		3.04 ± 0.11^c	0.99	71			2.1
Non-infused in EVOH	25	Zero	1.54 ± 0.06^a	0.99	144	25.33 ± 1.14^a	0.99	1
	35		2.22 ± 0.08^b	0.99	104			1.4
	45		2.97 ± 0.1^c	0.99	73			1.9

different letters denote significant statistical difference ($p < 0.05$) among a column for a fixed parameter

7.3.4 Time-temperature-tolerance approach

The approach presented above takes into account the deteriorations during isothermal conditions. However, in real situations, temperature variations are very rampant, for example, during the supply chain part of the product. As can be seen from the results of previous sections, higher temperatures cause greater deterioration of the product; hence, even short residence in higher temperatures will abruptly reduce the shelf life of the product.

The approach presented accounts for cumulative spoilages caused when the product is subjected to high temperatures at all the possible places before actually going to the shelf of the consumer (Dattatreya et al., 2007). A realistic shelf-life of gingerols infused candied mango product has been calculated as per the method detailed by (Dattatreya et al., 2007) and presented in **Table 7.4**.

Table 7.4: Illustration of quality degradation and shelf life estimates on isothermal storage conditions based on the time-temperature-tolerance approach

Product's location	Location temperature, °C	Shelf life (SL)* at location, days	Residence time (RT) in location, days	$SL_i\ RT_i^{-1}$ (fraction of quality lost in this step)	$\Sigma\ (SL_i\ RT_i^{-1})$, a cumulative fraction of quality lost
1. Manufacturing facility					
a. Packaging area	35	173	2	0.012	0.012
b. Warehouse	25	338	30	0.089	0.100
2. Supply chain	45	119	10	0.084	0.184
3. Shopping facility	25	338	90	0.266	0.451
4. Consumer's storehouse	25	338	60	0.178	0.628

*considering shelf life of gingerols infused product stored in MET package calculated on multivariate approach

The product reaches its end of shelf life when the cumulative fraction of quality loss becomes 1. At the starting point, when the product is kept for 2 days at the packaging area (35 °C), a fraction of quality loss is 0.012. Thereafter it is kept for 30 days at the warehouse (25 °C), where another 0.089 fraction is lost. Then it is subjected to 45 °C during supply chain for 10 days, where the fraction of quality loss is 0.084. After that, it reaches the shop where it is kept for another 90 days at 25 °C, and the fractional loss at this stage is 0.266. Finally, it spends 60 days on the shelf of the consumer (25 °C), where another 0.178 fractions of quality are lost. After 60 days, the cumulative fraction of

TH-2388_136107006

quality loss is 0.628. At this stage, the consumer still has 125 days (= 0.372 × 338 days) to consume the product, if stored at 25 ^{o}C considering 338 days of shelf life as calculated in the previous section. The method illustrated could be used to estimate a realistic shelf life of a product and can help in developing a suitable supply chain and storage strategies for a specific product.

7.4 Summary

The shelf life of candied mango product was enhanced by infusing the natural antioxidants of ginger under accelerated shelf-life test, conducted in different conditions of temperature and packaging materials. Specific key results are:

- Incorporation of gingerols into candied mango matrix not only improved the sensory profiles but also significantly retarded the browning inducing reactions as quantified in a range of quality parameters, and hence, substantially enhanced shelf-life in all conditions of storage.
- Based on univariate kinetics of all the individual quality parameters, β-carotene was found to be the most abruptly changing parameter.
- Multivariate data analysis of storage data was a more holistic approach as it considered the cumulative effect of deteriorations caused by multiple spoilage factors.
- Highest predicted shelf life of gingerols infused candied mango product was 338 days as against 155 days for the non-infused product when stored in MET pouch at 25 ^{o}C.
- Slower deterioration of all quality parameters was observed in MET than EVOH packaging material for both products in all temperatures.
- In order to verify the preciseness of calculated predictions, the actual shelf life of control samples stored in EVOH pouches at 25 ^{o}C was compared with predicted values. Chemometrics based 144 days of predicted shelf life was close to the actual shelf life of 142 days but varied significantly from 185 days of univariate kinetics based predictions.
- Thus infusion of gingerols could be employed as a green method for enhancement of shelf life of food products. Moreover, the proposed chemometrics based approach can be employed as a simple and low cost method for predicting the accurate shelf life of industrial food products.

TH-2386_136107006

Chapter 8

Conclusions, significant impact of thesis and scope for future work

The chapter summarizes the significant conclusions drawn from the dissertation work and presents the most significant impact of the thesis. It also gives an outline of the avenues that can be explored for carrying out future work.

TH-2386_136107006

TH-2386_136107006

8.1 Overall conclusions

The thesis presents a comprehensive database of chemical and physical properties of nine different varieties of ginger collected from various parts of northeast India. The relative abundance of the mineral contents follows a trend of K > Na > Mg > Ca > Fe > P > Mn > Zn > Cr > Ni > Cu for all the varieties. Ethanol is the best solvent for extracting maximum phenolic (0.96 mg g^{-1}) and flavonoids (1.52 mg g^{-1}) content from dried rhizomes. *Sungro-sung* variety from Nagaland contains highest content of 6- gingerol (2.2 % dry basis). This is the highest 6-gingerol content found in any ginger variety reported so far. Chemometric tools have clearly segregated *Sungro-sung* followed by *Arunachal Pradesh local* varieties as the best in terms of overall antioxidant as well as nutritive potential. The correlation study within all the properties reveals a strong inverse relationship among the average size with major bioactive constituents of oleoresin and volatile oils of all the varieties. Thus the physical size of ginger rhizome is a rapid, inexpensive and non-destructive parameter which can be used for screening a pool of ginger varieties for commercial production of volatile oil and oleoresin.

Considering the promising antioxidant prospects of *Sungro-sung* variety, a single step green process for supercritical CO_2 ($SCCO_2$) extraction coupled with fractionation of dry ginger for simultaneous production of gingerols rich oleoresin and volatile oil is developed and reported for the first time. The optimization and scale-up validation of this process is achieved in three stages. Firstly, $SCCO_2$ extraction conditions are optimized for the highest yield of mixture extract containing a maximum amount of volatile oil components and major actives [sum of (6+8+10) gingerols and 6-shogaol]. The optimum process variables under laboratory scale conditions are: average ginger particle size 253 µm, extraction temperature 40 °C, pressure 276 bar, flow rate 30 g min^{-1} and 153 min of extraction time. This results in 8.6 % yield of a mixture extract having 37.97 wt % of major actives and 28.3 wt % of volatile oil. In the second stage of experiments, these conditions are scaled up fifty folds on a commercial scale unit. This results in an improvement of 0.1% yield with an increase of 0.34 wt % and 1.2 wt % in the major actives and volatile oil contents, respectively. Finally, the optimum conditions of online $SCCO_2$ fractionation (coupled with same optimum extraction conditions) results in 5.95 % oleoresin yield, which is 96.15 % pure and 51.2 wt % of major actives, in a separator operating at 175 bar/40 °C. Simultaneously, 2.71 % yield of volatile oil (95.94 % pure) is recovered in another separator (40 bar/40 °C). The yield and quality of oleoresin and volatile oil extracted from conventional methods are also compared.

The extracted gingerols rich oleoresin is a lipid based non-water soluble extract of ginger. Hence, its application in food supplements and the pharmaceutical sector is also highly limited because of poor water solubility of its actives, which severely reduces its oral bioavailability and hence efficacy. Hence, a stable formulation of ginger oleoresin for the delivery of ginger actives targeted for food applications involving water based manufacturing processes is developed for the first time. The optimized formulation contains 27 wt % surfactant (Gelucire 44/14), 11 wt % co-surfactant (Transcutol 90), 46.5 wt % of ginger oleoresin and 15.5 wt % of Capryol 90 as oily vehicle. The dispersion prepared from the formulation had globules in nano range (<50 nm) and stable until 24 h.

The anhydrous formulation is found to be stable in a long term storage for 90 days in an accelerated condition (40 ^{o}C and 75 % RH). The developed formulation also shows 95.4 % enhancement in water-dispersibility of ginger actives over pure oleoresin. Besides, it also demonstrates commercial potential for food additive applications with integrated properties of food flavouring, shelf-life enhancer, and nutraceutical agent. Thus, the developed formulation with food-grade carriers for the delivery of ginger actives of ginger oleoresin has a promising commercial potential, especially in the food industries.

Consequently, the developed formulation is utilized for developing a ginger actives incorporated fruit based ready to eat nutraceutical food product. The collated effects of undertaking various process improvement for developing gingerols infused candied mango product measures result in drastic improvement of the nutritional and functional quality of candied mango slices. Gingerols infusion into mango matrix not only improves the functional quality, but the ginger mango flavor synergy is also responsible for the enhancement in the overall sensory scores of the developed product. Further optimization of the subsequent candying process for maximum sugar and water loss of mango slices is responsible for minimizing the processing losses of natural bioactive compounds present in the mango.

Thereafter, the process conditions optimized at bench scale are scaled-up 200 folds and augmented with commercial-scale vacuum drying at a commercial scale candy manufacturing facility. The manufactured product has 85.6, 76.8, 60.2 % retention in β-carotene, total phenolics, vitamin C, respectively, along with minor color difference and significant improvement in the sensory scores over fresh mango. A strong agreement is achieved in mass transfer properties and all the quality characteristics between bench and

TH-2388_13107006

manufacturing scale experiments imply excellent scalability and repeatability of the complete process. The current study also results in significant enhancement of vitamin C, TPC, and β-carotene contents by 233.3, 131.8 and 42.7 %, respectively along with superior color, sensory and textural properties, compared with a similar popular commercial candied mango product. In addition, the drastic reduction of overall process time from 143 to just 30 h is also recorded. Thus, the developed manual process is a very simple candy manufacturing process, which can be used to develop a commercial process of other popular fruit/vegetable based candied products, particularly targeted with reducing postharvest losses.

Finally, the quantitative effect of gingerols incorporation in shelf qualities of the developed candied mango product are investigated. Incorporation of nano-encapsulated gingerols into candied mango matrix not only improves the sensory profiles but also significantly retards the browning inducing reactions as quantified in a range of associated quality parameters. This intervention substantially enhances the shelf-life in all conditions of storage. Based on univariate kinetics of all the individual quality parameters, β-carotene is found to be the most abruptly changing parameter. Multivariate analysis of storage data is a more holistic approach as it considers the cumulative effect of deteriorations caused by multiple spoilage factors. Highest predicted shelf life of gingerols infused candied mango product is 338 days as against 155 days for the non-infused product when stored in MET pouch at 25 ^{o}C. Slower deterioration of all quality parameters is observed in MET over EVOH packaging material for both products in all temperatures. In order to verify the preciseness of calculated predictions, the actual shelf life of control samples stored in EVOH pouches at 25 ^{o}C are compared with predicted values. Chemometrics based 144 days of predicted shelf life is close to the actual shelf life of 142 days but varies significantly from 185 days of univariate kinetics based predictions. Thus, infusion of gingerols is a green method for enhancement of shelf life of food products. Moreover, the proposed multivariate data analysis based approach can be employed as a simple and low cost method for predicting the accurate shelf life of industrial food products.

8.2 Significant impact of the thesis work

- The thesis work contributes to the current state of art by identifying *Sungro-sung* variety of ginger from Nagaland having the highest 6-gingerol content in the world.

- The thesis reports a supercritical CO_2 based green process for simultaneous separation of volatile oil and gingerols enriched oleoresin from dry ginger, for the first time. In addition, the scaled-up validated process produced extracts which are comparable to the highest quality commercial ginger volatile oil and oleoresin available in market.
- Infusion of gingerols in fruit matrix for development of ginger flavored nutraceutical candied mango has been reported for the first time. In addition, infusion of gingerols also proved to be a credible tool for substantial enhancement of nutraceutical, sensory and shelf-life of the product.

8.3 Scope for future work

1. The developed single step green extraction process involving $SCCO_2$ also produces volatile oil of very high quality. Unlike developing the application of gingerols enriched extract in the current study, the application of volatile oil can be studied to develop an aromatic ready to use food product like flavoured tea dip sachets product.
2. The stability study of Ar-curcumene and its correlation with γ-curcumene can be studied for further standardizing CO_2 extract.
3. Following the results of formulation development for gingerols enrich extract, spray drying process can be utilized for developing microencapsulated gingerols enriched oleoresin dried powder. Various optimization studies can be conducted for developing ready to eat dry mix food products specific to dried oleoresin powder.
4. The developed water soluble formulation could be analyzed for bioavailability enhancement, cell line studies in order to validate its pharmacological application potential.
5. Various bench and commercial scale studies can be undertaken for optimizing the rheology and flow properties of the developed formulation in order to make it suitable for filling in soft/hard gelatin capsules for applications as a health supplement/drug product.

TH-2388_13610006

References

TH-2386_136107006

TH-2386_136107006

References

Additives, E.P.O.F., Food, N.S.A.T., 2015. Scientific Opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbitan tristearate (E 436) as food additives. EFSA Journal 13, 4152.

Adel, P.R.S., Prakash, J., 2010. Chemical composition and antioxidant properties of ginger root (Zingiber officinale). Journal of Medicinal Plants Research 4, 2674-2679.

Adsare, S.R., Bellary, A.N., Sowbhagya, H.B., Baskaran, R., Prakash, M., Rastogi, N.K., 2016. Osmotic treatment for the impregnation of anthocyanin in candies from Indian gooseberry (Emblica officinalis). Journal of Food Engineering 175, 24-32.

Badalyan, A.G., Wilkinson, G.T., Chun, B.-S., 1998. Extraction of Australian ginger root with carbon dioxide and ethanol entrainer. The Journal of supercritical fluids 13, 319-324.

Badola, R., Panjagari, N.R., Singh, R.R.B., Singh, A.K., Prasad, W.G., 2018. Effect of clove bud and curry leaf essential oils on the anti-oxidative and anti-microbial activity of burfi, a milk-based confection. Journal of Food Science and Technology 55, 4802-4810.

Bakht, M.A., Alajmi, M.F., Alam, P., Alam, A., Alam, P., Aljarba, T.M., 2014. Theoretical and experimental study on lipophilicity and wound healing activity of ginger compounds. Asian Pacific Journal of Tropical Biomedicine 4, 329-333.

Balachandran, S., Kentish, S., Mawson, R., Ashokkumar, M., 2006. Ultrasonic enhancement of the supercritical extraction from ginger. Ultrasonics sonochemistry 13, 471-479.

Barker, S.A., Yap, S.P., Yuen, K.H., McCoy, C.P., Murphy, J.R., Craig, D.Q.M., 2003. An investigation into the structure and bioavailability of α-tocopherol dispersions in Gelucire 44/14. Journal of Controlled Release 91, 477-488.

Bartley, J.P., Foley, P., 1994. Supercritical fluid extraction of australian-grown ginger (Zingiber officinale). Journal of the Science of Food and Agriculture 66, 365-371.

Bellary, A.N., Indiramma, A.R., Prakash, M., Baskaran, R., Rastogi, N.K., 2016. Anthocyanin infused watermelon rind and its stability during storage. Innovative Food Science & Emerging Technologies 33, 554-562.

Bellary, A.N., Rastogi, N.K., 2014. Effect of Selected Pretreatments on Impregnation of Curcuminoids and Their Influence on Physico-chemical Properties of Raw Banana Slices. Food and Bioprocess Technology 7, 2803-2812.

Bellary, A.N., Sowbhagya, H.B., Rastogi, N.K., 2011. Osmotic dehydration assisted impregnation of curcuminoids in coconut slices. Journal of Food Engineering 105, 453-459.

Bhattarai, S., Tran, V.H., Duke, C.C., 2001. The Stability of Gingerol and Shogaol in Aqueous Solutions. Journal of Pharmaceutical Sciences 90, 1658-1664.

Caparino, O.A., Nindo, C.I., Tang, J., Sablani, S.S., Chew, B.P., Mathison, B.D., Fellman, J.K., Powers, J.R., 2017. Physical and chemical stability of Refractance Window®–dried mango (Philippine 'Carabao' var.) powder during storage. Drying Technology 35, 25-37.

Cappa, C., Lavelli, V., Mariotti, M., 2015. Fruit candies enriched with grape skin powders: physicochemical properties. LWT - Food Science and Technology 62, 569-575.

Chambin, O., Jannin, V., 2005. Interest of Multifunctional Lipid Excipients: Case of Gelucire® 44/14. Drug Development and Industrial Pharmacy 31, 527-534.

Chakraborty, R., Samanta, R., 2015. A lphonso Mango Enrichment with A loe Vera (A loe barbadensis) by Sequential Drying: Optimization, Kinetics and Quality Evaluation. Journal of food processing and preservation 39, 2698-2709.

Chakraborty, S., Rao, P.S., Mishra, H.N., 2016. Changes in Quality Attributes During Storage of High-Pressure and Thermally Processed Pineapple Puree. Food and Bioprocess Technology 9, 768-791.

Chen, H.-H., Chung, C.-C., Wang, H.-Y., Huang, T.-C., 2011. Application of Taguchi method to optimize extracted ginger oil in different drying conditions. IPCBEE May, 7-9.

Chen, H., Rustagi, S., Diep, E., Langrish, T.A.G., Glasser, B.J., 2018. Scale-up of fluidized bed drying: Impact of process and design parameters. Powder Technology 339, 8-16.

Chen, Y., Lin, H., Li, Y., Lin, M., Chen, J., 2019. Non-enzymatic browning and the kinetic model of 5-hydroxymethylfurfural formation in residual solution of vinegar soaked-soybean. Industrial Crops and Products 135, 146-152.

TH-2388_13170006

Carmona-Jiménez, Y., García-Moreno, M.V., Igartuburu, J.M., Garcia Barroso, C., 2014. Simplification of the DPPH assay for estimating the antioxidant activity of wine and wine by-products. Food Chemistry 165, 198-204.

Crank, J., 1975. The mathematics of diffusion. Clarendon Press.

Ceylan, Z., Meral, R., Kose, Y. E., & Cavidoglu, I. (2019). Wheat germ oil nanoemulsion for oil stability of the cooked fish fillets stored at 4 °C. Journal of Food Science and Technology.

Das, A., Das, C., Golder, A.K., 2016. Evaluation of Physico-Chemical Properties of Dried Aloe Gel: Comparison among Hot Air, Microwave-Assisted and Hybrid Drying Processes. The Natural Products Journal 6, 219-226.

Das, A.B., Goud, V.V., Das, C., 2017. Extraction of phenolic compounds and anthocyanin from black and purple rice bran (Oryza sativa L.) using ultrasound: A comparative analysis and phytochemical profiling. Industrial Crops and Products 95, 332-341.

Da Porto, C., Decorti, D., Natolino, A., 2014. Separation of aroma compounds from industrial hemp inflorescences (Cannabis sativa L.) by supercritical CO_2 extraction and on-line fractionation. Industrial Crops and Products 58, 99-103.

Date, A.A., Nagarsenker, M.S., 2007. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. International Journal of Pharmaceutics 329, 166-172.

Dattatreya, A., Etzel, M.R., Rankin, S.A., 2007. Kinetics of browning during accelerated storage of sweet whey powder and prediction of its shelf life. International Dairy Journal 17, 177-182.

Dermesonlouoglou, E., Chalkia, A., Taoukis, P., 2018. Application of osmotic dehydration to improve the quality of dried goji berry. Journal of food engineering 232, 36-43.

Derossi, A., Mastrandrea, L., Amodio, M.L., de Chiara, M.L.V., Colelli, G., 2016. Application of multivariate accelerated test for the shelf life estimation of fresh-cut lettuce. Journal of Food Engineering 169, 122-130.

Eleazu, C., Amadi, C., Iwo, G., Nwosu, P., Ironua, C., 2013. Chemical Composition and Free Radical Scavenging Activities of 10 Elite Acces-sions of Ginger (Zingiber officinale Roscoe). J Clinic Toxicol 3, 155.

Faisal, W., Ruane-O'Hora, T., O'Driscoll, C.M., Griffin, B.T., 2013. A novel lipid-based solid dispersion for enhancing oral bioavailability of Lycopene – In vivo evaluation using a pig model. International Journal of Pharmaceutics 453, 307-314.

George, J.M., Senthamizh Selvan, T., Rastogi, N.K., 2016. High-pressure-assisted infusion of bioactive compounds in apple slices. Innovative Food Science & Emerging Technologies 33, 100-107.

George, J.M., Sowbhagya, H.B., Rastogi, N.K., 2017. Effect of high pressure pretreatment on drying kinetics and oleoresin extraction from ginger. Drying Technology, 1-10.

Ghasemzadeh, A., Jaafar, H.Z.E., Rahmat, A., 2015. Optimization protocol for the extraction of 6-gingerol and 6-shogaol from Zingiber officinale var. rubrum Theilade and improving antioxidant and anticancer activity using response surface methodology. BMC Complementary and Alternative Medicine 15, 258.

Giannakourou, M., Strati, I.F., Kriebardis, A.G., Mantanika, V., Poulis, S., Zoumpoulakis, P., Sinanoglou, V.J., 2019. Shelf Life Extension and Quality Improvement of Cucumber Slices Impregnated in Infusions of Edible Herbs. Analytical Letters, 1-15.

Giraldo, G., Talens, P., Fito, P., Chiralt, A., 2003. Influence of sucrose solution concentration on kinetics and yield during osmotic dehydration of mango. Journal of Food Engineering 58, 33-43.

Golfomitsou, I., Mitsou, E., Xenakis, A., Papadimitriou, V., 2018. Development of food grade O/W nanoemulsions as carriers of vitamin D for the fortification of emulsion based food matrices: A structural and activity study. Journal of Molecular Liquids 268, 734-742.

Govindarajan, V.S., Connell, D.W., 1983. Ginger — chemistry, technology, and quality evaluation: Part 1. C R C Critical Reviews in Food Science and Nutrition 17, 1-96.

Guiamba, I., Ahrné, L., Khan, M.A.M., Svanberg, U., 2016. Retention of β-carotene and vitamin C in dried mango osmotically pretreated with osmotic solutions containing calcium or ascorbic acid. Food and Bioproducts Processing 98, 320-326.

Haldar, S., Mishra, H.N., Majumdar, G.C., 2016. Optimization of Oleoresin Extraction from Curcuma longa L. Using RSM and Determination of Equilibrium Constant. Journal of Food Processing and Preservation 40, 1188-1198.

TH-2388_13177006

Hazarika, D.J., Kakoti, M., 2013. Study on the Indigenous Varieties of Ginger of Golaghat District (Assam), and its Economic Viability as Aroma Ingredients. Journal of Natural Products and Plant Resources. 3, 24-29.

Huang, W., Su, H., Wen, L., Shao, A., Yang, F., Chen, G., 2018. Enhanced anticancer effect of Brucea javanica oil by solidified self-microemulsifying drug delivery system. Journal of Drug Delivery Science and Technology 48, 266-273.

Ippoushi, K., Azuma, K., Ito, H., Horie, H., Higashio, H., 2003. [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. Life Sciences 73, 3427-3437.

Ismail, R.K., Lingamallu, J., 2012. An Overview on Inventions Related to Ginger Processing and Products for food and Pharmaceutical Applications. Recent Patents on Food, Nutrition & Agriculture 4, 31-49.

Jain, S., Jain, A.K., Pohekar, M., Thanki, K., 2013. Novel self-emulsifying formulation of quercetin for improved in vivo antioxidant potential: Implications for drug-induced cardiotoxicity and nephrotoxicity. Free Radical Biology and Medicine 65, 117-130.

Jha, S.N., Rai, D.R., Shrama, R., 2012. Physico-chemical quality parameters and overall quality index of apple during storage. Journal of Food Science and Technology 49, 594-600.

Jiménez-Hernández, J., Estrada-Bahena, E.B., Maldonado-Astudillo, Y.I., Talavera-Mendoza, Ó., Arámbula-Villa, G., Azuara, E., Álvarez-Fitz, P., Ramírez, M., Salazar, R., 2016. Osmotic dehydration of mango with impregnation of inulin and piquin-pepper oleoresin. LWT - Food Science and Technology.

Joshi, R.P., Negi, G., Kumar, A., Pawar, Y.B., Munjal, B., Bansal, A.K., Sharma, S.S., 2013. SNEDDS curcumin formulation leads to enhanced protection from pain and functional deficits associated with diabetic neuropathy: An insight into its mechanism for neuroprotection. Nanomedicine: Nanotechnology, Biology and Medicine 9, 776-785.

Kaushik, N., Kaur, B.P., Rao, P.S., Mishra, H.N., 2014. Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (Mangifera indica cv. Amrapali). Innovative Food Science & Emerging Technologies 22, 40-50.

Kaushik, N., Rao, P.S., Mishra, H.N., 2018. Comparative analysis of thermal-assisted high pressure and thermally processed mango pulp: Influence of processing, packaging, and storage. Food Science and Technology International 24, 15-34.

Khoo, S.-M., Humberstone, A.J., Porter, C.J.H., Edwards, G.A., Charman, W.N., 1998. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. International Journal of Pharmaceutics 167, 155-164.

Khan, M.A.M., Ahrné, L., Oliveira, J.C., Oliveira, F.A.R., 2008. Prediction of water and soluble solids concentration during osmotic dehydration of mango. Food and Bioproducts Processing 86, 7-13.

Kiran, C.R., Chakka, A.K., Amma, K.P.P., Menon, A.N., Kumar, M.M.S., Venugopalan, V.V., 2013. Influence of Cultivar and Maturity at Harvest on the Essential Oil Composition, Oleoresin and [6]-Gingerol Contents in Fresh Ginger from Northeast India. Journal of Agricultural and Food Chemistry 61, 4145-4154.

Kou, X., Ke, Y., Wang, X., Rahman, M.R.T., Xie, Y., Chen, S., Wang, H., 2018. Simultaneous extraction of hydrophobic and hydrophilic bioactive compounds from ginger (Zingiber officinale Roscoe). Food Chemistry 257, 223-229.

Krumreich, F.D., Borges, C.D., Mendonça, C.R.B., Jansen-Alves, C., Zambiazi, R.C., 2018. Bioactive compounds and quality parameters of avocado oil obtained by different processes. Food Chemistry 257, 376-381.

Kubra, I.R., Rao, L.J.M., 2012. An Impression on Current Developments in the Technology, Chemistry, and Biological Activities of Ginger (Zingiber officinale Roscoe). Critical Reviews in Food Science and Nutrition 52, 651-688.

Kuentz, M., 2011. Oral self-emulsifying drug delivery systems, from biopharmaceutical to technical formulation aspects. Journal of Drug Delivery Science and Technology 21, 17-26.

Latona, D.F., Oyeleke, G.O., Olayiwola, O.A., 2012 Chemical Analysis of Ginger Root. Journal of Applied Chemistry (IOSRJAC) 1, 47-49.

Lei, H., Li, X., Wei, Q., Zhou, R., Wu, J., 2016. Extraction and purification of ginger essential oil by supercritical carbon dioxide combined with macroporous resin method. Current Topics in Nutraceutical Research 14, 59-66.

Link, J.V., Tribuzi, G., Laurindo, J.B., 2017. Improving quality of dried fruits: A comparison between conductive multi-flash and traditional drying methods. LWT 84, 717-725.

TH-2388_136107006

Lu, L., Wu, J., Wei, L., Wu, F., 2016. Temperature dependence of aggregated structure of β-carotene by absorption spectral experiment and simulation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 169, 116-121.

Madamba, P.S., Driscoll, R.H., Buckle, K.A., 1996. The thin-layer drying characteristics of garlic slices. Journal of Food Engineering 29, 75-97.

Majumder, M., Shukla, A.K., Arunachalam, A., 2011. Agricultural Practices in Northeast India and Options for Sustainable Management, in: Lichtfouse, E. (Ed.), Biodiversity, Biofuels, Agroforestry and Conservation Agriculture. Springer Netherlands, Dordrecht, pp. 287-315.

Meral, R., Ceylan, Z., & Kose, S. (2019). Limitation of microbial spoilage of rainbow trout fillets using characterized thyme oil antibacterial nanoemulsions. Journal of Food Safety, 39(4), e12644.

Mukherjee, S., Mandal, N., Dey, A., Mondal, B., 2014. An approach towards optimization of the extraction of polyphenolic antioxidants from ginger (Zingiber officinale). Journal of Food Science and Technology 51, 3301-3308.

Naik, S.N., Lentz, H., Maheshwari, R.C., 1989. Extraction of perfumes and flavours from plant materials with liquid carbon dioxide under liquid—vapor equilibrium conditions. Fluid Phase Equilibria 49, 115-126.

Nair, K.P.P., 2013. 16 - The Chemistry of Ginger, The Agronomy and Economy of Turmeric and Ginger. Elsevier, Oxford, pp. 293-337.

Nambiar, S.S., Basu, A., Shetty, N.P., Rastogi, N.K., Prapulla, S.G., 2016. Infusion of fructooligosaccharide in Indian gooseberry (Emblica officinalis) fruit using osmotic treatment and its effect on the antioxidant activity of the fruit. Journal of Food Engineering 190, 139-146.

Ndawula, J., Kabasa, J.D., Byaruhanga, Y.B., 2004. Alterations in fruit and vegetable beta-carotene and vitamin C content caused by open-sun drying, visqueen-covered and polyethylene-covered solar-dryers. African health sciences 4, 125-130.

Niamprem, P., Rujivipat, S., & Tiyaboonchai, W. (2014). Development and characterization of lutein-loaded SNEDDS for enhanced absorption in Caco-2 cells. Pharmaceutical Development and Technology, 19(6), 735-742.

Ogino, M., Yakushiji, K., Suzuki, H., Shiokawa, K., Kikuchi, H., Seto, Y., Sato, H., Onoue, S., 2018. Enhanced pharmacokinetic behavior and hepatoprotective function of ginger extract-loaded supersaturable self-emulsifying drug delivery systems. Journal of Functional Foods 40, 156-163.

Oriani, V.B., Alvim, I.D., Consoli, L., Molina, G., Pastore, G.M., Hubinger, M.D., 2016. Solid lipid microparticles produced by spray chilling technique to deliver ginger oleoresin: Structure and compound retention. Food Research International 80, 41-49.

Otunola, G.A., Oloyede, O.B., Oladiji, A.T., Afolayan, A.J., 2010. Comparative analysis of the chemical composition of three spices–Allium sativum L. Zingiber officinale Rosc. and Capsicum frutescens L. commonly consumed in Nigeria. African Journal of Biotechnology 9, 6927-6931.

Pandotra, P., Viz, B., Ram, G., Gupta, A.P., Gupta, S., 2015. Multi-elemental profiling and chemo-metric validation revealed nutritional qualities of Zingiber officinale. Ecotoxicology and Environmental Safety 114, 222-231.

Panigrahi, K.C., Patra, C.N., Jena, G.K., Ghose, D., Jena, J., Panda, S.K., Sahu, M., 2018. Gelucire: A versatile polymer for modified release drug delivery system. Future Journal of Pharmaceutical Sciences 4, 102-108.

Parmar, N., Singla, N., Amin, S., Kohli, K., 2011. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids and Surfaces B: Biointerfaces 86, 327-338.

Parthasarathy, V.A., Chempakam, B., Zachariah, T.J., 2008. Chemistry of Spices. CABI.

Pattnaik, P.K., Kar, D., Kuanar, A., Sahu, A., Mishra, B., 2016. Molecular & Biochemical characterization of selected elite accessions of ginger. Biocatalysis and Agricultural Biotechnology 8, 130-138.

Paula, J.T., Aguiar, A.C., Sousa, I.M.O., Magalhães, P.M., Foglio, M.A., Cabral, F.A., 2016. Scale-up study of supercritical fluid extraction process for Baccharis dracunculifolia. The Journal of Supercritical Fluids 107, 219-225.

Pawar, N., Pai, S., Nimbalkar, M., Dixit, G., 2011. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. Food Chemistry 126, 1330-1336.

Perry, R.H., Green, D.W., 1999. Perry's chemical engineers' handbook. McGraw-Hill Professional.

Pickardt, C., Neidhart, S., Griesbach, C., Dube, M., Knauf, U., Kammerer, D.R., Carle, R., 2009. Optimisation of mild-acidic protein extraction from defatted sunflower (Helianthus annuus L.) meal. Food Hydrocolloids 23, 1966-1973.

Pouton, C.W., 2006. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. European journal of pharmaceutical sciences 29, 278-287.

Pouton, C.W., 1997. Formulation of self-emulsifying drug delivery systems. Advanced Drug Delivery Reviews 25, 47-58.

Pouton, C.W., 2000. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. European Journal of Pharmaceutical Sciences 11, S93-S98.

Povh, N.P., Marques, M.O.M., Meireles, M.A.A., 2001. Supercritical CO_2 extraction of essential oil and oleoresin from chamomile (Chamomilla recutita [L.] Rauschert). The Journal of Supercritical Fluids 21, 245-256.

Pradhan, R.C., Meda, V., Rout, P.K., Naik, S., Dalai, A.K., 2010. Supercritical CO_2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. Journal of Food Engineering 98, 393-397.

Radha krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., Sukumar, M., 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. International Journal of Food Microbiology 171, 32-40.

Raghavendra, S.N., Rastogi, N.K., Raghavarao, K.S.M.S., Tharanathan, R.N., 2004. Dietary fiber from coconut residue: effects of different treatments and particle size on the hydration properties. European Food Research and Technology 218, 563-567.

Rahman, H., Karuppaiyan, R., Kishore, K., Denzongpa, R., 2009. Traditional practices of ginger cultivation in Northeast India.

Rahath Kubra, I., Kumar, D., Rao, L.J.M., 2013. Effect of microwave-assisted extraction on the release of polyphenols from ginger (Zingiber officinale). International Journal of Food Science & Technology 48, 1828-1833.

Raina, V.K., Kumar, A., Aggarwal, K.K., 2005. Essential Oil Composition of Ginger (Zingiber officinale Roscoe) Rhizomes from Different Place in India. Journal of Essential Oil Bearing Plants 8, 187-191.

Ranganna, S., 1986. Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education.

Rastogi, N., Raghavarao, K., Niranjan, K., Knorr, D., 2002. Recent developments in osmotic dehydration: methods to enhance mass transfer. Trends in Food Science & Technology 13, 48-59.

Ravi Kiran, C., Chakka, A.K., Padmakumari Amma, K.P., Nirmala Menon, A., Sree Kumar, M.M., Venugopalan, V.V., 2013. Essential oil composition of fresh ginger cultivars from North-East India. Journal of Essential Oil Research 25, 380-387.

Reverchon, E., 1997. Supercritical fluid extraction and fractionation of essential oils and related products. The Journal of Supercritical Fluids 10, 1-37.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. The Journal of Supercritical Fluids 38, 146-166.

Rout, P.K., Naik, M.K., Naik, S.N., Goud, V.V., Das, L.M., Dalai, A.K., 2009. Supercritical CO_2 Fractionation of Bio-oil Produced from Mixed Biomass of Wheat and Wood Sawdust. Energy & Fuels 23, 6181-6188.

Rout, P.K., Naik, S.N., Rao, Y.R., 2008. Subcritical CO_2 extraction of floral fragrance from Quisqualis indica. The Journal of Supercritical Fluids 45, 200-205.

Rózek, A., Achaerandio, I., Güell, C., López, F., Ferrando, M., 2010. Use of commercial grape phenolic extracts to supplement solid foodstuff. LWT - Food Science and Technology 43, 623-631.

Rezaei, A., Fathi, M., Jafari, S.M., 2019. Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. Food Hydrocolloids 88, 146-162.

Said, P.P., Arya, O.P., Pradhan, R.C., Singh, R.S., Rai, B.N., 2015. Separation of Oleoresin from Ginger Rhizome Powder Using Green Processing Technologies. Journal of Food Process Engineering 38, 107-114.

Salea, R., Veriansyah, B., Tjandrawinata, R.R., 2017. Optimization and scale-up process for supercritical fluids extraction of ginger oil from Zingiber officinale var. Amarum. The Journal of Supercritical Fluids 120, 285-294.

Sanwal, S.K., Rai, N., Singh, J., Buragohain, J., 2010. Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (Zingiber officinale Roscoe). Scientia Horticulturae 124, 280-285.

Sehwag, S., Upadhyay, R., Das, M., 2018. Optimization and multivariate accelerated shelf life testing (MASLT) of a low glycemic whole jamun (Syzygium cumini L.) confection with tailored quality and functional attributes. Journal of food science and technology 55, 4887-4900.

Semwal, R.B., Semwal, D.K., Combrinck, S., Viljoen, A.M., 2015. Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 117, 554-568.

TH-2388_136107006

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F.J., Khar, R.K., Ali, M., 2007. Development and bioavailability assessment of ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics 66, 227-243.

Shahidi, F., Zhong, Y., 2015. Measurement of antioxidant activity. Journal of Functional Foods 18, 757-781.

Shakeel, F., Haq, N., Alanazi, F.K., Alsarra, I.A., 2013. Impact of various nonionic surfactants on self-nanoemulsification efficiency of two grades of Capryol (Capryol-90 and Capryol-PGMC). Journal of Molecular Liquids 182, 57-63.

Shukla, A., Goud, V.V., Das, C., 2019a. Antioxidant potential and nutritional compositions of selected ginger varieties found in northeast India. Industrial Crops and Products 128, 167-176.

Shukla, A., Naik, S.N., Goud, V.V., Das, C., 2019b. Supercritical CO_2 extraction and online fractionation of dry ginger for production of high-quality volatile oil and gingerols enriched oleoresin. Industrial Crops and Products 130, 352-362.

Shukla, Y., Singh, M., 2007. Cancer preventive properties of ginger: A brief review. Food and Chemical Toxicology 45, 683-690.

Silva, F.M., Silva, C.L.M., 1999. Colour changes in thermally processed cupuaçu (Theobroma grandiflorum) puree: critical times and kinetics modelling. International Journal of Food Science & Technology 34, 87-94.

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kumari, R., Dhaka, N., 2013. Potential applications of antioxidants – A review. Journal of Pharmacy Research 7, 828-835.

Singh, R.P., Gangadharappa, H.V., Mruthunjaya, K., 2018. Phytosome complexed with chitosan for gingerol delivery in the treatment of respiratory infection: In vitro and in vivo evaluation. European Journal of Pharmaceutical Sciences 122, 214-229.

Sowbhagya, H.B., Smitha, S., Sampathu, S.R., Krishnamurthy, N., Bhattacharya, S., 2005. Stability of water-soluble turmeric colourant in an extruded food product during storage. Journal of Food Engineering 67, 367-371.

Srinivasan, K., 2017. Ginger rhizomes (Zingiber officinale): A spice with multiple health beneficial potentials. PharmaNutrition 5, 18-28.

Syamila, M., Gedi, M.A., Briars, R., Ayed, C., Gray, D.A., 2019. Effect of temperature, oxygen and light on the degradation of β-carotene, lutein and α-tocopherol in spray-dried spinach juice powder during storage. Food Chemistry 284, 188-197.

Tao, Y.Q., Shi, Y., Zhang, S.M., Dong, X.Y., Gao, P.P., Wang, J.H., Zhao, N., Ruan, H.H., Zhao, H., 2013. A new process of extracting oleoresin ginger from ginger by critical-state liquid CO_2, Advanced Materials Research, pp. 212-216.

Taoukis, P.S., Labuza, T.P., Saguy, I.S., 1997. Kinetics of Food Deterioration and Shelf-Life Prediction, Handbook of Food Engineering Practice. CRC Press, pp. 365-407.

Thayumanavan, B., Sadasivam, S., 1984. Physicohemical basis for the preferential uses of certain rice varieties. Plant Foods for Human Nutrition 34, 253-259.

Telfser, A., Gómez Galindo, F., 2019. Effect of reversible permeabilization in combination with different drying methods on the structure and sensorial quality of dried basil (Ocimum basilicum L.) leaves. LWT 99, 148-155.

Tian, H.-X., Zhang, Y.-J., Chen, C., Qin, L., Xiao, L.-Z., Ma, H.-R., Yu, H.-Y., 2019. Effects of Natural Ingredients on the Shelf Life of Chicken Seasoning. Food Chemistry.

Tolve, R., Condelli, N., Caruso, M.C., Genovese, F., Di Renzo, G.C., Mauriello, G., Galgano, F., 2018. Preparation and characterization of microencapsulated phytosterols for the formulation of functional foods: Scale up from laboratory to semi-technical production. Food Research International.

Upadhyay, R., Mishra, H.N., 2015. A multivariate approach to optimise the synergistic blend of oleoresin rosemary (Rosmarinus officinalis L.) and ascorbyl palmitate added into sunflower oil. International Journal of Food Science & Technology 50, 974-981.

Upadhyay, R., Sehwag, S., Mishra, H.N., 2017. Chemometric approach to develop frying stable sunflower oil blends stabilized with oleoresin rosemary and ascorbyl palmitate. Food chemistry 218, 496-504.

Varakumar, S., Umesh, K.V., Singhal, R.S., 2017. Enhanced extraction of oleoresin from ginger (Zingiber officinale) rhizome powder using enzyme-assisted three phase partitioning. Food Chemistry 216, 27-36.

Vásquez-Caicedo, A.L., Schilling, S., Carle, R., Neidhart, S., 2007. Impact of packaging and storage conditions on colour and β-carotene retention of pasteurised mango purée. European Food Research and Technology 224, 581-590.

Vieira da Silva, B., Barreira, J.C.M., Oliveira, M.B.P.P., 2016. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. Trends in Food Science & Technology 50, 144-158.

Wang, Q., Yang, Q., Cao, X., Wei, Q., Firempong, C.K., Guo, M., Shi, F., Xu, X., Deng, W., Yu, J., 2018. Enhanced oral bioavailability and anti-gout activity of [6]-shogaol-loaded solid lipid nanoparticles. International journal of pharmaceutics 550, 24-34.

Warisnoicharoen, W., Lansley, A.B., Lawrence, M.J., 2000. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. International Journal of Pharmaceutics 198, 7-27.

Wibowo, S., Buvé, C., Hendrickx, M., Van Loey, A., Grauwet, T., 2018. Integrated science-based approach to study quality changes of shelf-stable food products during storage: A proof of concept on orange and mango juices. Trends in Food Science & Technology 73, 76-86.

Wibowo, S., Grauwet, T., Gedefa, G.B., Hendrickx, M., Van Loey, A., 2015. Quality changes of pasteurised mango juice during storage. Part II: Kinetic modelling of the shelf-life markers. Food Research International 78, 410-423.

Wibowo, S., Grauwet, T., Santiago, J.S., Tomic, J., Vervoort, L., Hendrickx, M., Van Loey, A., 2015. Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. Food chemistry 187, 140-151.

Wohlmuth, H., Smith, M.K., Brooks, L.O., Myers, S.P., Leach, D.N., 2006. Essential Oil Composition of Diploid and Tetraploid Clones of Ginger (Zingiber officinale Roscoe) Grown in Australia. Journal of Agricultural and Food Chemistry 54, 1414-1419.

Xu, Y., Wang, Q., Feng, Y., Firempong, C.K., Zhu, Y., Omari-Siaw, E., Zheng, Y., Pu, Z., Xu, X., Yu, J., 2016. Enhanced oral bioavailability of [6]-Gingerol-SMEDDS: Preparation, in vitro and in vivo evaluation. Journal of functional foods 27, 703-710.

Yeung, A.W.K., Mocan, A., Atanasov, A.G., 2018. Let food be thy medicine and medicine be thy food: A bibliometric analysis of the most cited papers focusing on nutraceuticals and functional foods. Food Chemistry 269, 455-465.

Yeh, H.-y., Chuang, C.-h., Chen, H.-c., Wan, C.-j., Chen, T.-l., Lin, L.-y., 2014. Bioactive components analysis of two various gingers (Zingiber officinale Roscoe) and antioxidant effect of ginger extracts. LWT - Food Science and Technology 55, 329-334.

Yingngam, B., Brantner, A., 2018. Boosting the essential oil yield from the rhizomes of cassumunar ginger by an eco-friendly solvent-free microwave extraction

combined with central composite design. Journal of Essential Oil Research 30, 409-420.

Yonei, Y., Ōhinata, H., Yoshida, R., Shimizu, Y., Yokoyama, C., 1995. Extraction of ginger flavor with liquid or supercritical carbon dioxide. The Journal of Supercritical Fluids 8, 156-161.

Yudthavorasit, S., Wongravee, K., Leepipatpiboon, N., 2014. Characteristic fingerprint based on gingerol derivative analysis for discrimination of ginger (Zingiber officinale) according to geographical origin using HPLC-DAD combined with chemometrics. Food Chemistry 158, 101-111.

Zancan, K.C., Marques, M.O., Petenate, A.J., Meireles, M.A.A., 2002. Extraction of ginger (Zingiber officinale Roscoe) oleoresin with CO 2 and co-solvents: a study of the antioxidant action of the extracts. The Journal of supercritical fluids 24, 57-76.

Indian Institute of Technology Guwahati

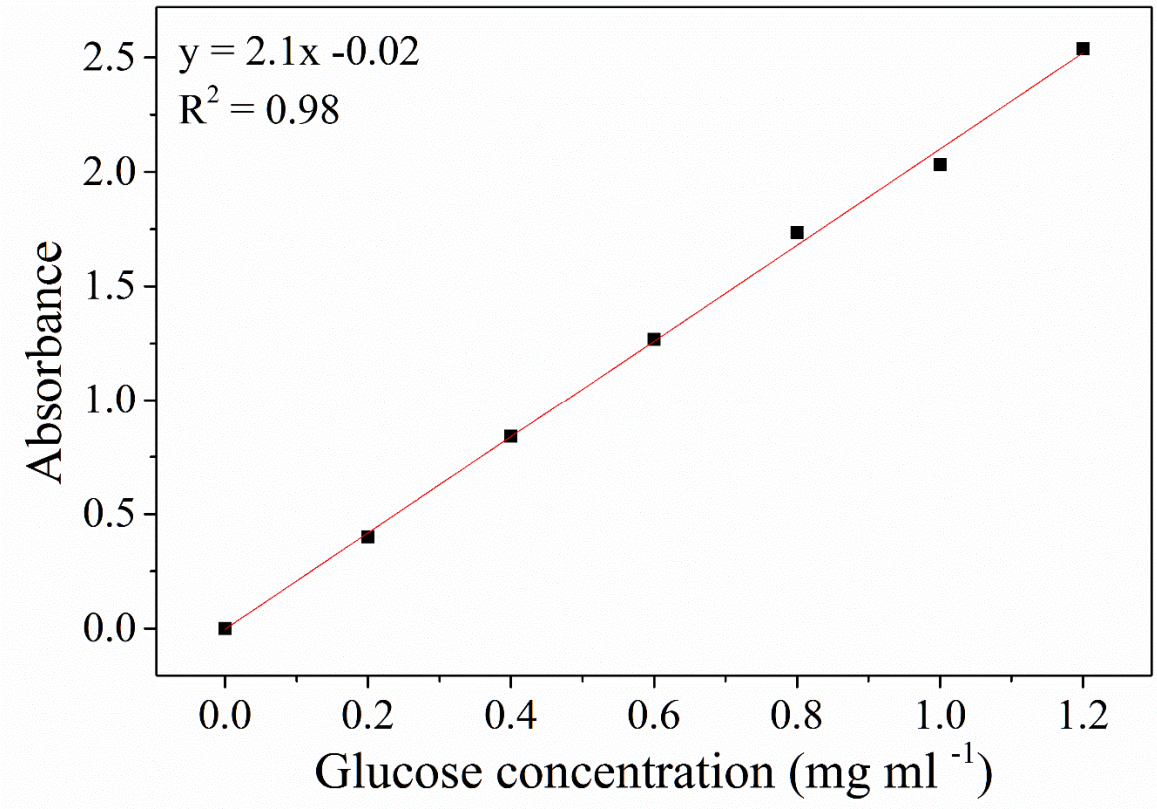
TH-2388_136107006

Appendix and research output

TH-2386_136107006

TH-2386_136107006

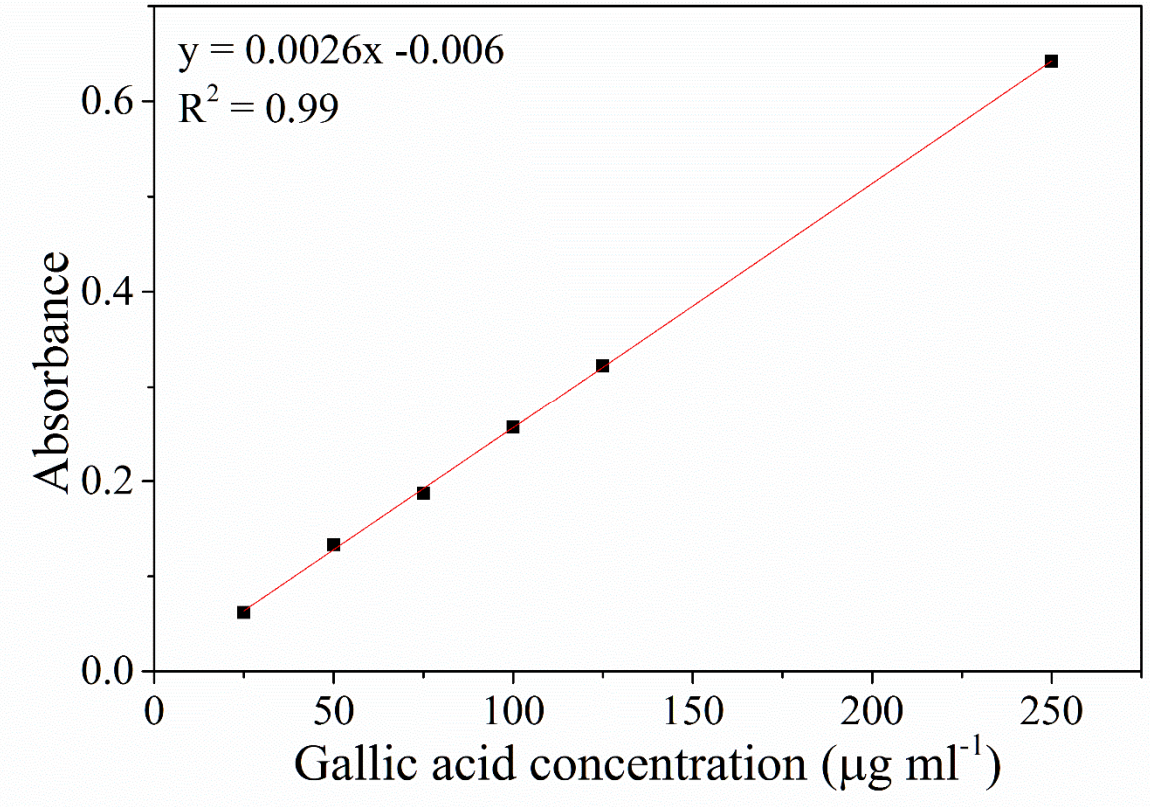
Appendix 1



Standard glucose calibration graph

dian Institute of Technology Guwahati

Appendix 2

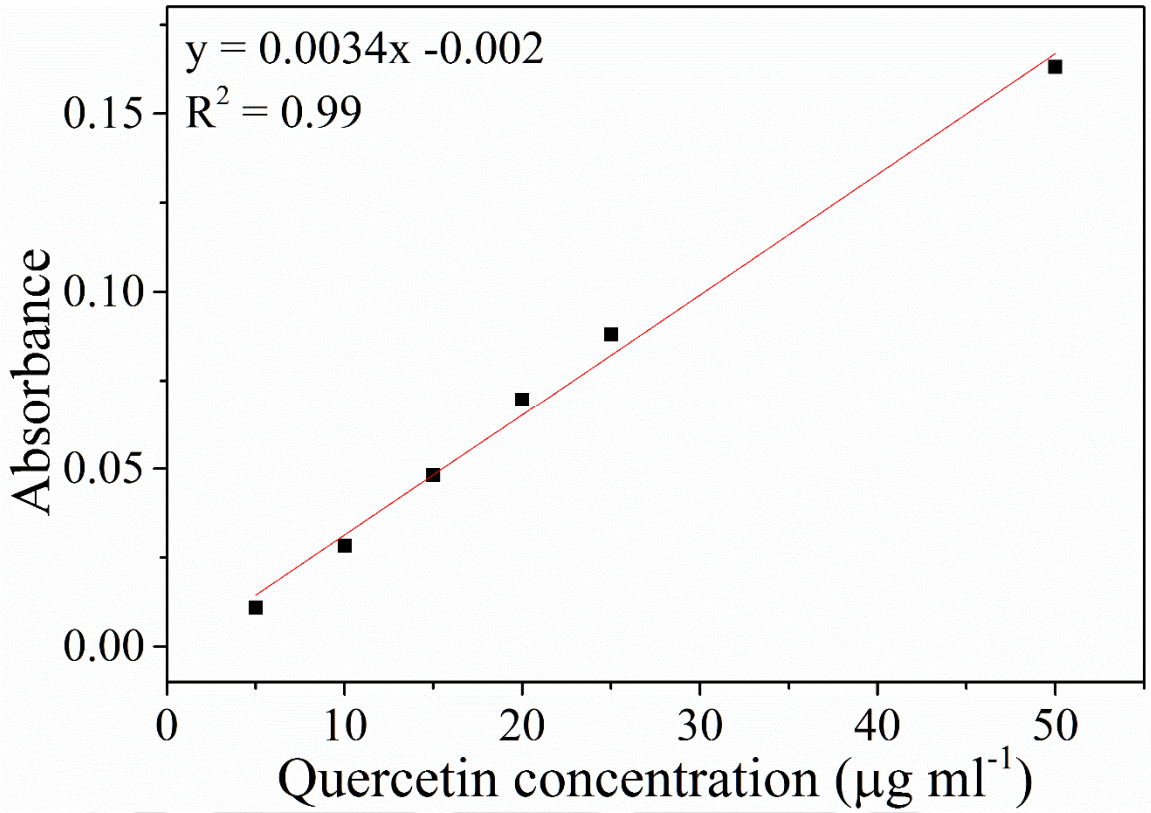


Standard Gallic acid calibration graph

an Institute of Technology Guwa

TH-2388_13137006

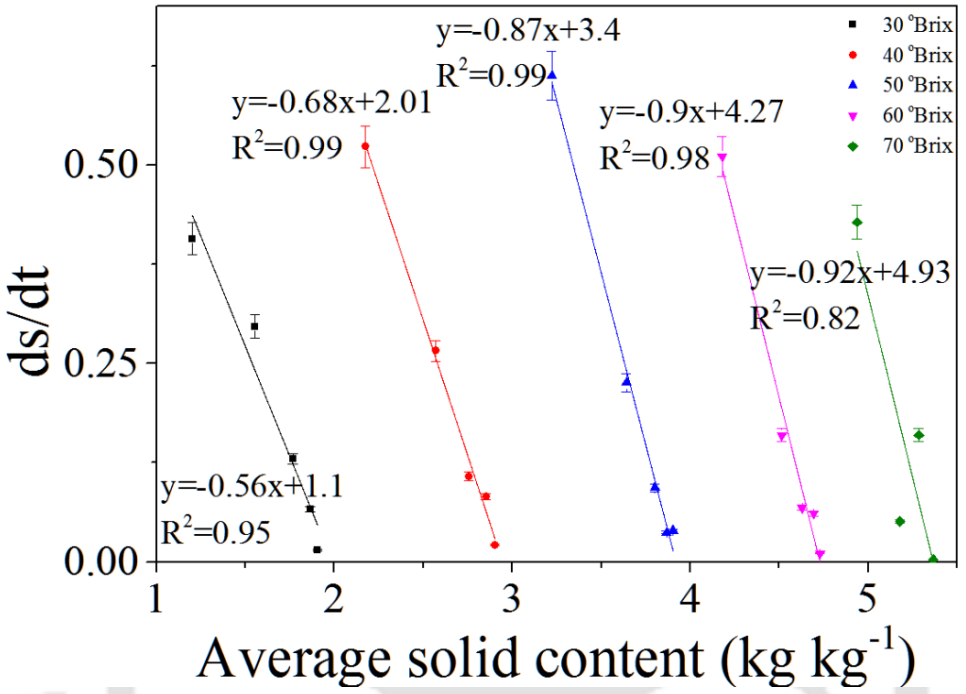
Appendix 3

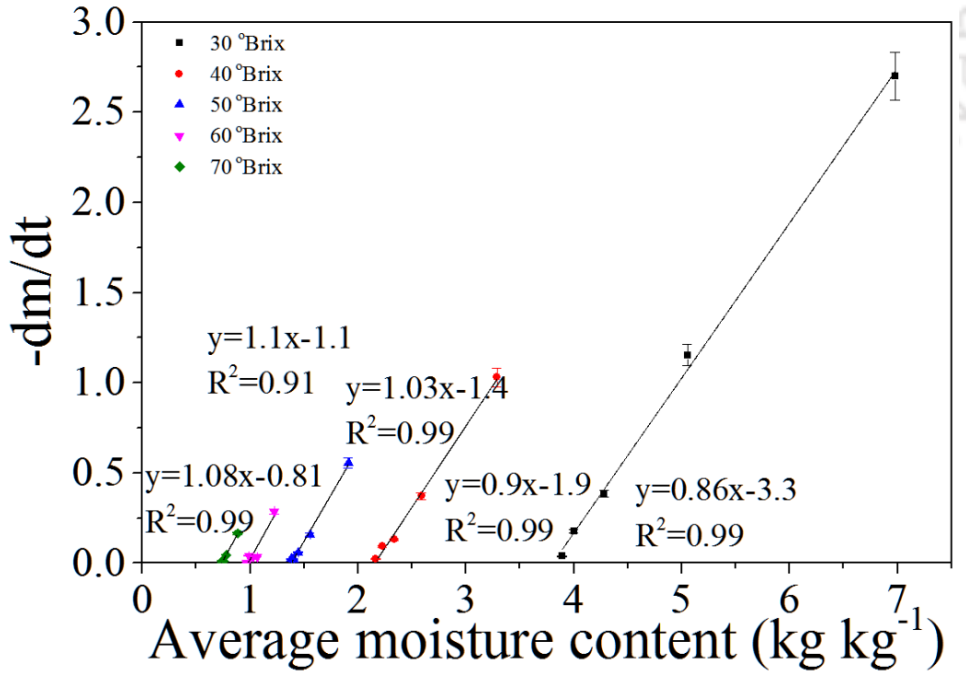


Standard Quercetin calibration graph

an Institute of Technology Guwa

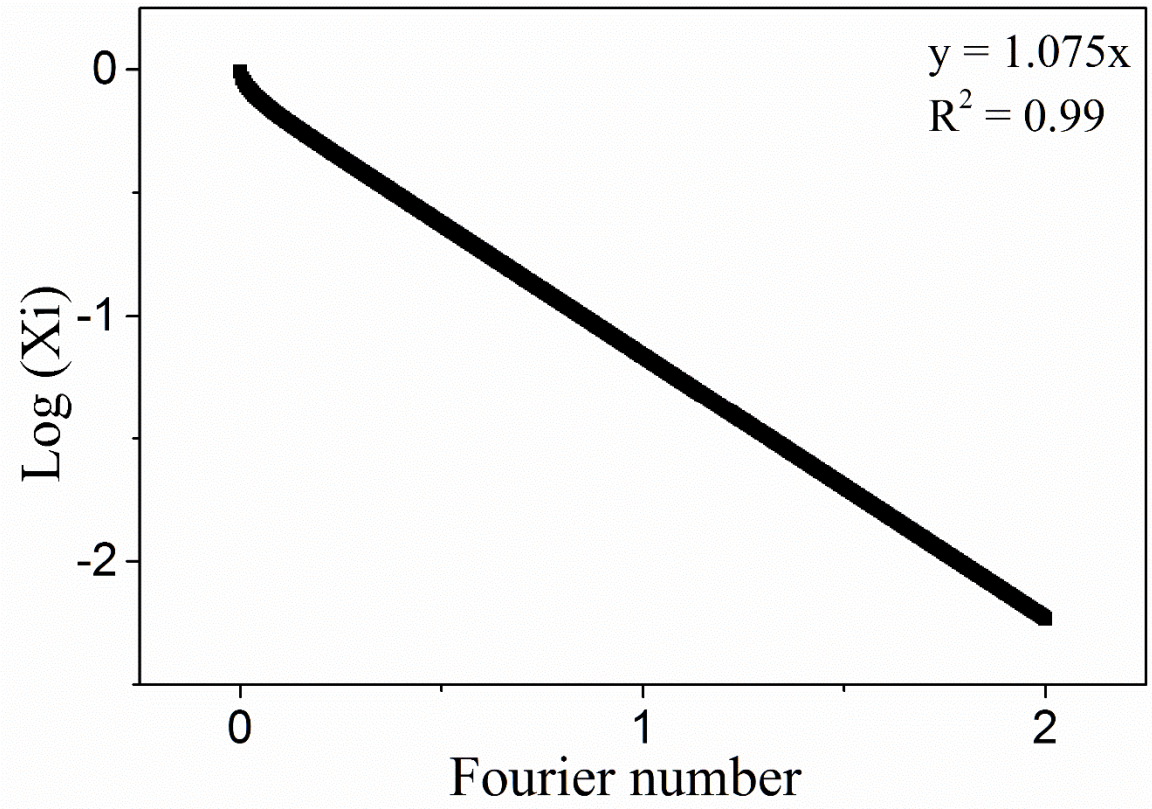
Appendix 4 (a)





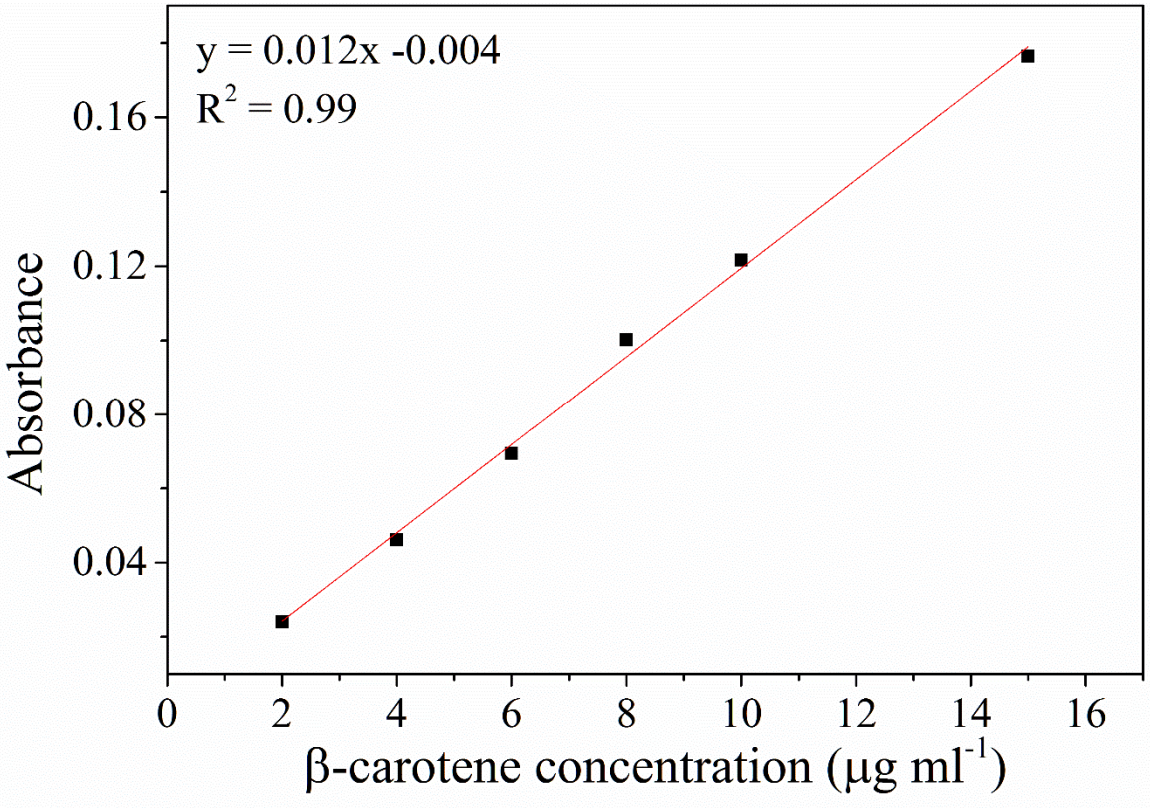
Average solids and moisture content plots

Appendix 4 (b)



Theoretical diffusion coefficient curve

Appendix 5



Standard β-carotene calibration graph

an Institute of Technology Guwa

TH-2388_136187006

Appendix 6

Error analysis

Step 1: Calculation of sample mean

$$\mu_x = \frac{\sum_{i=1}^{n} x_i}{n} \quad \text{(A1)}$$

Step 2: calculation of standard deviation

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \mu_x)^2}{n-1}} \quad \text{(A2)}$$

Step 3: calculation of standard error (SE)

$$SE = \frac{s}{\sqrt{n}} \quad \text{(A3)}$$

Sample calculation

$SCCO_2$ Extraction parameters	**Oleoresin yield %**	**wt % major actives**	**wt % volatile oil**
	8.4	36.3	27.4
Pressure : 300 (bar)	7.9	35.2	27.3
Flow rate (g min^{-1}) : 40	8.6	35.8	26.8
Time (min) : 180	8.2	39.8	24.9
	8.1	34.5	25.4
Mean	**8.2**	**36.3**	**26.4**
Standard deviation	**0.2**	**1.8**	**1**
Standard error	**0.1**	**0.8**	**0.5**

TH-2386_136107006

Research output:

A. Journal publications

1. Shukla, A., Das, C., & Goud, V. V. (2020). Design of a carrier system for gingerols enriched oleoresin tailored for food applications. *Food and Bioproducts Processing*, *124*, 296-306.
2. Shukla, A., Das, C., & Goud, V. V. (2020). Infusion of gingerols into candied mango enhances shelf-life by inhibiting browning and associated quality parameters during storage. *Food Chemistry, 316,* 126354.
3. Shukla, A., Shukla, R. S., Das, C., & Goud, V. V. (2019). Gingerols infusion and multi-step process optimization for enhancement of color, sensory and functional profiles of candied mango. *Food Chemistry, 300*, 125195.
4. Shukla, A., Naik, S. N., Goud, V. V., & Das, C. (2019). Supercritical CO_2 extraction and online fractionation of dry ginger for production of high-quality volatile oil and gingerols enriched oleoresin. *Industrial Crops and Products, 130*, 352-362.
5. Shukla, A., Goud, V. V., & Das, C. (2019). Antioxidant potential and nutritional compositions of selected ginger varieties found in northeast India. *Industrial Crops and Products, 128*, 167-176.

B. Conference

1. “Industrial manufacturing of fruit candy: Optimization of overall process” in Technical Session "Food and Bio-Process Engineering" at International conference, Emerging Trends in Agricultural & Food Engineering (ETAE) during 27 – 30 December 2016, organized by Agricultural and Food Engineering Dept., IIT Kharagpur. The address was selected for “Best Oral Presentation” category.
2. “Promising Commercial Prospects of Some Indigenous Ginger Varieties of North East India” in Technical Session "Functional, nutraceutical and health foods" at national conference, Trends in Food Processing Technology (TIFPT) during 9 – 10 February 2017, organized by Department of Food Engineering and Technology, Tezpur University.
3. “Process optimization for industrial manufacturing of fruit based nutraceutical candy systems” in Technical Session "Nutrition, nutraceutical and functional

foods" at International conference, Bioprocessing India (BPI) during 9 – 11 December 2017, organized by Department of Biosciences and Bioengineering, IIT Guwahati.

4. "A single step green process for industrial scale production of high-quality oleoresin and volatile oil from ginger" at Indo-Japan Bilateral Symposium on Future Perspective of Bioresource Utilization in North-Eastern Region (IJBS 17) during 1 – 4 February 2018, organized by Department of Chemical Engineering, IIT Guwahati.
5. "Separation of high value bio-actives from herbs and plants of Northeast India" at TEQIP workshop on Recent Advances in Food Engineering in North-East India during 17-19 December 2020, organized by Department of Chemical Engineering, IIT Guwahati.

TH-2388_13197006

Abhishek Shukla

Resecher scholar

Department of Chemical engineering

Indian Institute of technology, Guwahati

Phone: 86380-88242

E-Mail: abhishekshukla@iitg.ac.in

insatiableabhi@gmail.com

Educational Details:

- PhD in Chemical Engineering from Indian Institute of Technology, Guwahati, Assam, India
- M. Tech. in Agricultural Processing and Food Engineering from Chaudhary Charan Singh Haryana Agricultural University
- Tech in Agricultural Engineering from Chaudhary Charan Singh Haryana Agricultural University

Professional experience

IIT Delhi

Center for Rural Development and Technology — Senior Research Fellow

Key Functional Areas

Process development for extraction of essential oil, oleoresin, pigments and antioxidants from herbs, spices and underutilized seeds using conventional and supercritical fluids

Patanjali Food & Herbal Park, Haridwar — Research Engineer

New Product Development Department & Herbal Extraction Unit

Key Functional Areas

- R & D and process scale-up studies for new generation nutraceuticals and cosmeceuticals
- Product and process improvement/standardization of existing line of food and herbal products