DEVELOPMENT AND OPTIMIZATION OF MICROWAVE ASSISTED PROCESS FOR EXTRACTION OF NUTMEG MACE ESSENTIAL OIL

by

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(2014 – 18 – 102)

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DECLARATION

I, hereby declare that this thesis entitled **"DEVELOPMENT AND OPTIMIZATION OF MICROWAVE ASSISTED PROCESS FOR EXTRACTION OF NUTMEG MACE ESSENTIAL OIL"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to
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LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree Celsius
%	:	Percentage
&	:	And
/	:	Per
D	:	Diameter
et al.	:	and others
etc.	:	Etcetera
GHz	:	Giga Hertz
g	:	gram
$g.l^{-1}$	:	gram per litre
$g.mg^{-1}.day^{-1}$	:	gram per milli gram per day
h	:	hour
Hz	:	Hertz
H_2O_2	:	Hydrogen peroxide
i.e.	:	that is
I.U	:	International Unit
K.C.A.E.T	:	Kelappaji College of Agricultural Engineering and Technology
Kcal	:	kilo calorie
kWh	:	kilo Watt hour
MAE	:	Microwave Assisted Extraction
mg	:	Milli gram
MHz	:	Mega Hertz
min	:	Minute (s)
ml	:	Milli Litre
mm	:	Milli Meter
mPas	:	Milli Pascal Second
MT	:	Metric Tonne
p	:	Probability

RDA	:	Recommended Dietary Allowance
RF	:	Radio Frequency
s	:	Second (s)
Sl.	:	Serial
t	:	Tonne
V	:	Volt
W	:	Watt
Wb	:	Wet basis
$W.g^{-1}$	:	Watt per gram
μ	:	Micro
λ	:	Lamda

CHAPTER-1

Introduction

CHAPTER I

INTRODUCTION

Spices which are obtained from plant or vegetable products or mixtures of both are used in whole or ground form for cooking, mainly for providing flavour, aroma and pungency to food. This low volume high value products are known to have disease inhibiting and health promoting properties. They have been in use since ancient times for their carminative, anti-inflammatory anti-flatulent properties. The active principles in the spices may help in smooth digestion through increasing intestinal system function and stimulating excessive secretion of gastro-intestinal enzymes inside the stomach. According to the latest report of the International Organization for Standardization (ISO), there are about 109 spices grown in different parts of the world. The highest producer, consumer and exporter of spices in the world is India. According to Spices Board, 52 spices are grown in the country. During 2013-14, total spices production in the country was estimated to be 0.58 million tonnes, in an area of around 0.31 million hectares (Anon., 2010). Among the spices grown in India, few of them grown commercially, and remaining grown on a small scale in gardens. But spices have good international market potential or are in great demand and are even imported (clove, nutmeg, cinnamon, long pepper, sage, thyme, marjoram, asafetida etc.). Five major spices i.e. black pepper, cardamom, chillies, ginger and turmeric contributes around 75-89 per cent of the total annual Indian foreign exchange earnings (Pruthy, 2001).

Spices are heterogeneous collections of a wide variety of volatile and non-volatile basic dietary additives. The oil of the plant known as "Essential oil" are the volatile components distilled from the aromatic plant materials which possess characteristic flavour and taste. The essential oil represent the essence or active elements of plants due to the presence of aroma compounds which are oily in nature. They are called volatile or ethereal oils as they evaporate when exposed to atmosphere at ambient temperatures. Demand and price of essential oils and herbal products are

increasing constantly in national and international markets due to strong pro-consumer movement. Essential oils contribution in the world of fragrance and flavour industry is about 17 per cent. Extent of usage of essential oils is 55-60 per cent for flavours in food industry, 15-21 per cent for fragrances in cosmetic / perfumery industry, 10-20 per cent as starting material for isolation of components, 5-10 per cent as active substances in pharmaceutical preparations and 2-5 per cent for natural products. Estimated production of perfumery raw material is around 5000 t/annum valued at Rs. 400 crores in India (Skaria *et al.,* 2007). About 90 per cent of India's requirement of essential oils is met from indigenous production and remaining from import.

Essential oils are generally extracted by distillation. Distillation may be defined as separation of components from a mixture of two or more liquids by virtue of difference in their vapour pressure. There are three systems of distillation- hydro, steam and hydro-steam distillation. Other processes include solvent extraction, expression, absolute oil extraction, resin tapping, and cold pressing. The main disadvantages when conventional methods of distillation were followed are: low quality of final product such as loss of some volatile notes, low extraction efficiency and loss of unsaturated ester compounds through thermal or hydrolytic effects (Ferhat *et al.,* 2006). Besides, these processes also requires high extraction times and energy consumption. However, in order to overcome the disadvantages or to reduce the extraction time, improve the extraction yield, enhance the quality of extracts and to reduce operational costs latest technologies such as microwave assisted extraction, pressurized solvent extraction, supercritical fluid extraction and ultrasound assisted extraction etc. have been sought.

Microwave energy could be used effectively to mediate the extraction of essential oil in place of steam or water heating in order to introduce its inherent advantages. As in the case of microwave heating of food materials, the internal heating of the already present water within the plant material by the microwaves leads to the rupture of the glands and odoriferous receptacles freeing the essential oil which is then

evaporated by the in-situ water of the plant material. The water then evaporated could then be passed through a condenser outside the microwave cavity where it is condensed. It has been found that the use of microwaves for extraction of active components could result in enhanced performance in terms of quality and quantity such as high extraction efficiency, less extraction time and increased yield with quality of the extracted oil superior to that of other conventional methods due to the mild conditions (Lucchesi *et al*., 2004). Besides, microwave extraction may be classified as a green technology and is energy efficient. The process control is also easy.

Since microwave heating is a volumetric process in which heating is through kinetic effects, biomaterials respond differently with the microwave energy. Therefore, it is important that the process parameters of extraction needs to be optimized for each plant materials.

Tree spices like nutmeg, clove, cinnamon and tamarind are important spices grown in India, especially in South India. Nutmeg is an evergreen tree spice is an important spice crop of India, and its cultivation is showing an increasing trend. The area and production of nutmeg in India during 2014-15 is 19,000 hectares and 12,780 tonnes, respectively out of which Kerala accounts for 97.15 per cent of the area and 98 per cent of total production (Anon., 2010).

Nutmeg (*Myristica fragrans* Houtt.,) is unique among spice plants as the donor of two distinct spices; nutmeg and mace. Fruit is composed of three parts: the pericarp or husk, the mace and the seed. The pericarp is removed and the mace which envelopes the shell is peeled off. The ratio of dried nutmeg to dried mace is approximately 20:3. Nutmeg mace, the dried aril surrounding the kernel is the basic raw material for the production of nutmeg mace oil. Nutmeg oil generally extracted by steam distillation is a potential ingredient in food, pharmaceutical and cosmetic industries. Nutmeg and its derivative nutmeg oil are being used across the world as inevitable flavours in the preparation of soups, meat products, sauces, baked foods, confectioneries and puddings, seasonings of meat and vegetables, ice-creams and desserts. Nutmeg oil is

an essential flavouring ingredient in milk dishes and punches, soft drinks, beverages, colas, canned foods and liquors of which form the mainstay of contemporary diet. Medicinally nutmeg oil is found to possess stimulative and carminative properties. The main constituents of nutmeg oil i.e. myristicin, elemicin and iso elemicin when used in aroma form, acts as stress relievers. Also, nutmeg oil find application in cosmetics, men's perfume and toiletries. They also possess insecticidal, antibacterial, antifungal, anticarcinogenic and antioxidant properties (Cho *et al*., 2007).

Some recently published studies have utilized the microwave energy for extraction of essential oil from various spices. Since microwaves heat the biomaterial through kinetic effects and is a volumetric heating process, the plant materials respond differently to the action of microwaves. Therefore, the process parameters leading to the efficient extraction and quality oil needs to be optimized for each material extracted using microwave assisted technology. In this context, it may be noted that such optimization studies pertaining to nutmeg oil has not been found reported.

Considering the above facts a study was undertaken on **"Development and optimization of microwave assisted process for extraction of nutmeg mace essential oil"** with the following objectives:

- To develop a system for microwave assisted extraction of volatiles from nutmeg mace.
- Evaluation of the developed system towards extraction of essential oil and optimization of the process parameters.
- Characterization of the microwave assisted extracted nutmeg mace essential oil in comparison with hydro distilled oil.

CHAPTER II

REVIEW OF LITERATURE

This chapter deals with the review of research work reported on the scenario of nutmeg and benefits of nutmeg usage as a food ingredient. Reviews on application of microwave technology in extraction of essential oil and dielectric properties of oil has been elaborately presented.

2.1 NUTMEG

Nutmeg (*Myristica fragrans* Houtt.,) is one of the most important spices with high economic value. It is an evergreen aromatic tree cultivated in many tropical countries. The two spices nutmeg and mace are called twins because they form the both parts of the fruit "*Myristica fragrans*". The main difference between nutmeg and mace is that the former is the kernel inside the seed and the later consists of the vein-like threads that surrounds the dried fruit. Both are traditional flavorings used in the preparation of sweets like custards, cakes, desserts and other palatable dishes especially spinach, pasta, fish and quiche. These are used in beverages like coca cola as a flavoring agent. They should not be used in large quantities because they contain hallucinogens, and can be fatally toxic, however consumption of small quantities normally used in cooking are considered safe. Fresh mace is highly demanded than nutmeg in ground form (Nelson, 2013).

2.1.1 Global Scenario

Native of Indonesia, nutmeg tree grows there abundantly and is now cultivated in West Indies, India, Phillipines, Srilanka, Tropical America and Pacific Islands (Verghese, 2000). In India, the plant is grown in certain pockets of Kerala, Tamil Nadu, Goa, Karnataka, Maharashtra, North East India and Andamans. But the major producer and supplier of nutmeg and mace is Indonesia, which contributes around 80

per cent of world nutmeg. The next major contribution of nutmeg is from Greneda (20 per cent) followed by Srilanka, Trinidad and Tobago (Krishnamoorthy, 2000).

On a global scale, the annual growth rate in spices consumption is estimated at around 10 per cent. More than 120 countries in the world imports spices from India. From the past 25 years world trade in spices has shown a constantly increasing trend. Along with other horticultural crops, spice crop like nutmeg can be used for intensive agriculture under mixed farming systems. However, the export value of nutmeg and mace has increased up to 22 per cent. About 3275 tonnes of nutmeg and mace is exported to other countries with an earning of Rs. 91.8 crores. The major importers of nutmeg and mace are UAE, Vietnam, Singapore and USA (IISR, 2013).

Rodianawati *et al.* (2015) stated that in general Indonesian nutmeg quality is low, which results in low commodity price. About 55 per cent of the seeds are broken, and 77 per cent of mace that reaches the consumers is of Broken II quality. In order to increase the sale value of broken seeds of nutmeg, processing of nutmeg into essential oils, nutmeg's oleoresin, and nutmeg butter were carried out.

2.1.2 National Scenario

The area and production of nutmeg in India during 2014-15 is 19,000 hectares and 12,780 MT, respectively. The value added products of nutmeg are spice oils, mint products, oleoresins, curry powder/paste/condiments, and spice powders. These products contributed around 58 per cent in value towards total export earnings by spices from India. Nutmeg oil is one of the major spice oils which is getting exported and USA is the major importer of spice extracts followed by Germany, UK, South Korea and China. The two major competing countries in production and export of nutmeg and mace with India are Guinea and Sri Lanka (IISR, 2013).

2.1.3 State Scenario

In Kerala nutmeg cultivation is mainly concentrated in the areas of Thrissur, Ernakulam and Kottayam districts. The climatic conditions of Kerala suit for nutmeg

cultivation, and it is grown in homesteads of Kerala as an intercrop. The major variations in the varieties of nutmeg available in Kerala are plants varying in growth patterns, shape and size of the nuts, the quality and quantity of the mace etc. Mother trees should possess around 10,000 fruits per year regularly, with a nut weight of 10 g each and mace 1 g/fruit (Bavappa and Ruettimann, 1981). Male or female nutmeg trees can exist. When seeds are planted, the male plants will be around 50 per cent and quantity of production is quite low. Moreover, until flowering starts it is difficult to identifying whether the plant is male or female. Due to the above reason nutmeg plantation has become a risky job for farmers. Hence farmers should prefer vegetative propagated plants using budding or grafting techniques for nutmeg cultivation. At the same time, it should be noticed that male trees are needed for pollination. Male-female trees in the ratio of 1:10 results in an ideal plantation (Rethinam and Edison, 1991). Among the total production of nutmeg in India, Kerala accounts for 97.15 per cent of the area and 98 per cent of the total production (SBI, 2015).

2.1.4 Benefits of Nutmeg

Nutmeg has been used over thousands of years for various purposes. Joseph and Rachael (2003) reported that nutmeg has a variety of therapeutic properties and can also be used in a wide range of recipes. It is also used for insomnia, anxiety, calming muscle spasms, vomiting, nausea, indigestion, diarrhea, gout, joint pain, lowering blood pressure level, male infertility, impotence, improving concentration, lowering cholesterol, increasing blood circulation, toothaches etc.

2.1.4.1 Antimicrobial Effect

Escherichia coli O157:H7 is an enterohemorrhagic serotype of the bacteria *Escherichia coli*, and leads to illness, typically when consumed contaminated raw food including raw milk. Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhea, and also cause kidney failure. Takikawa *et al.* (2002) studied antimicrobial activity of nutmeg against *Escherichia coli* O157:H7. The solutions

obtained by homogenizing various spices (5 g each) at 25^{o}C for 10 min with 5 ml of 70 per cent ethyl alcohol, followed by centrifugation were used as spice extracts. A noticeable difference regarding their tolerance to nutmeg was observed between the O157 and non-pathogenic strains when the E. coli strains were incubated with each spice extract at concentrations of 0.01 per cent and 0.l per cent. The populations of the O157 strains were remarkably reduced whereas non-pathogenic strains could not be reduced.

Latha *et al.* (2005) stated that the essential oils obtained from nutmeg seeds are used in tonics. Also, they inhibit the growth of *Listeria monocytogenes* by abolishing the production of the bacterial extracellular protein, listeriolysin and the bacterial enzyme phospholipase. Nutmeg extract showed mild antibacterial activity against pathogenic staphylococci. Gupta *et al.* (2013) conducted a study on antioxidant and antimicrobial potential of nutmeg. Here, extracts of nutmeg were evaluated using disc diffusion method, to check antimicrobial activity against gram positive (*B. subtilis and S. aureus*), gram negative (*P. putida and P. aeruginosa*) bacteria and pathogenic fungi (*A. fumigatus, A. niger and A. flavus*). Also their minimum inhibitory concentrations (MIC) were determined. It was found that the extracts of nutmeg used in the present study possess substantial antimicrobial activity against the tested microorganism. Acetone extract of nutmeg has shown vigorous antimicrobial activity than all other extracts of nutmeg used in the study. High antioxidant and antimicrobial activity could be due to the presence of α-pinene, β-pinene, myrcene, 1, 8-cineole, carvacrol, terpinen-4-ol, eugenol and isoeugenol. This study strongly maintained the ethno-pharmacological importance of the nutmeg. Also, it was found that the antioxidant and antimicrobial activity possessed by nutmeg could be helpful in preventing or to reduce the progress of various oxidative stress-related diseases and infections by subtle harmful microorganisms.

2.1.4.2 Cytotoxic, Anticancer and Chemo Protective Effects

It is generally accepted that the body can be protected against chemical carcinogenesis or damage, especially during the starting phase, by inducing Phase I or Phase II drug metabolizing enzymes. Morita *et al.* (2003) studied hepatoprotective effect of myristicin from nutmeg on lipopolysaccharide/d-galactosamine-induced liver injury and concluded that in order to protect the body organs against carcinogenesis essential oils could be utilized. Similarly, nutmeg showed a powerful hepatoprotective activity against liver damage caused by certain chemicals. The protective activity was correlated with myristicin, a major constituent. Recently it was found that myristicin induces cytotoxicity in human neuroblastoma SK-N-SH cells by an apoptotic mechanism i.e. eliminating unhealthy and unnecessary cells (Lee *et al.*, 2005).

2.1.4.3 Nutritional and Health Benefits

Burdock (1995) stated that specific gravity of nutmeg mace oil at 25^{o}C falls within the range of 0.880 to 0.930, refractive index at 20^{o}C varies between 1.4740 and 1.4880 and solubility is 1:3 in 90 per cent ethanol.

About 30-55 per cent of the nutmeg seed consists of oils and 45-60 per cent consists of solid matter including cellulose matter. Two types of nutmeg oil are present:

- Essential oil
- Fixed oil

The essential oil also called as volatile oil account for 5-15 per cent and fixed oil account for 24-40 per cent (Forrest and Heacock, 1972; Abdullah *et al.*, 2010). The relative percentages of the different components will vary depending on the geographical origin of the nutmeg. Table 2.1 shows the analysis of nutrients per 100 g of ground mace spice.

Table 2.1. Nutritional value per 100 g of mace spice. (Agbogidi and Azagbaekwe, 2013).

Principle	Nutrient Value	Percentage of RDA
Energy	475 Kcal	24
Carbohydrates	50.50 g	39
Protein	6.71 g	12
Total Fat	32.38 g	162
Cholesterol	0 mg	0
Dietary Fiber	20.2 g	54
Vitamins		
Folates	76 µg	19
Niacin	1.350 mg	8
Pyridoxine	0.160 mg	12
Riboflavin	0.448 mg	34
Thiamin	0.312 mg	26
Vitamin-A	800 IU	27
Vitamin C	21 mg	35
Electrolytes		
Sodium	80 mg	5
Potassium	463 mg	10
Minerals		
Calcium	252 mg	25
Copper	2.467 mg	274
Iron	13.90 mg	174
Magnesium	163 mg	41
Manganese	1.500 mg	65
Phosphorus	110 mg	30
Zinc	2.15 mg	20

The major components of essential oil and their relative percentages are as follows:

Table 2.2. Major components of essential oil of nutmeg mace. (Takikawa *et al.*, 2002).

Sl.No	Component	Percentage (per cent)
1.	Sabinene or Camphene	50
2.	α-Pinene	20
3.	Dipentene	8
4.	d-Linalool	6
5.	d-Borneol	6
6.	i-Terpineol	6
7.	Geraniol	6
8.	Myristicin	4
9.	Safrole	0.6
10.	Eugenol	2
11.	iso Eugenol	2

Health benefits

Nutmeg helps in curing flatulence, diarrhea, and improves appetite as well. It is used in cough syrup which helps to cure the congestion resulting from cold. It is even helpful in aroma therapy (Gill, 1992; Iwu, 1993). The main properties of nutmeg are stimulating the brain, providing relief from stress and also nourishing mental activities. It improves blood circulation to brain, resulting in improved concentration and assimilation rate. However, it should be taken in small amounts, as too much can cause delirium (Hallstrom and Thuvander, 1997). As it stimulates the heart functions by increasing blood circulation, it can be considered as an excellent tonic for the cardiovascular system (Balick and Paul, 2000). The toxins can be removed using nutmeg oil and hence it acts as a great liver tonic. It dissolves kidney stones and hence

acts as an excellent remedy for kidney infections (Kasahara *et al*., 2005). The studies showed that nutmeg can cure insomnia and induce relaxation by increasing the level of serotonin (Pandey, 2005).

Nutmeg oil is used in treating bad breath (Barceloux, 2009). It helps to cure gum problems as well as toothaches due to its antiseptic nature. It is because of this peculiar property that the oil is even used in many kinds of toothpaste (Duke, 1994; Osemene *et al*., 2013).

Medicinal uses

Muscular pains and rheumatic pains of joints can be cured by doing massage with nutmeg oil (Pamplona-Roger, 1999). Nausea, gastritis and indigestion ailment can be relieved by consuming freshly prepared decoction with honey (Dorman *et al.,* 2000). Powdered seeds or decoction of the seeds of nutmeg were used in the treatment of diarrhea, carminative and rheumatism (Sofowora, 1993; Okoegwale and Omofezi, 2001). Nutmeg and its oil were being used as a traditional medicine in China and India for curing illness related to the nervous and digestive systems since ancient times. Myristicin and elemicin present in these spices not only stimulates brain but also acts as soothing agent (Maikhubu, 2006).

In order to remove the unpleasant odor of the various herbal preparations, nutmeg seed powder is added as a flavouring agent. Vomiting, nausea and flatulence can be treated by using decoction of the nutmeg. Stomach pain can be relieved by rubbing with nutmeg oil. Piles can be cured by applying grated nutmeg mixed with Vaseline (Agbogidi and Azagbaekwe, 2013).

2.2 MICROWAVES

Datta and Anantheswaran (2000) stated that microwaves are the electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz with a corresponding wavelength ranging from 1 m to 1 mm. Domestic microwave appliances operate generally at a frequency of 2450 MHz, while industrial microwave systems

operate at a frequency of 915 MHz and 2450 GHz. Microwaves are coherent and polarized in contrast to visible waves (apart from lasers). Based on the laws of optics and also on the type of material, microwaves can be reflected, absorbed or transmitted.

Guan *et al.* (2011) described the other advantages of microwave food processing such as uniformity in heating the product, low operation cost due to a drastic reduction in processing time, ease of operation, low maintenance, flavor and nutritional changes in food were very less and surface browning and crusting of the product can be prevented due to heating from inside.

Chandrasekaran *et al.* (2013) and Jermann *et al.* (2015) reported that microwaves found wide applications in the area of food processing such as drying, pasteurization, cooking and preservation of food materials. Apart from conventional thermal processing techniques heat is generated volumetrically throughout the product at faster rates in microwave heating. Solid and pumpable foods can be processed by means of microwaves effectively. This includes fluids containing large particles.

2.3 MICROWAVE HEATING

During microwave and thermal processing of honey changes pertaining to antioxidant activity and formation of 5-hydroxymethylfurfural (HMF) was observed by Kowalski (2013). Four types of honey (honeydew, lime, acacia and buckwheat) were analyzed in that study. The samples were subjected to the action of a microwave field (MW) with constant power of 1.26 $W.g^{-1}$ up to 6 min and also to conventional heating in a water bath (WB) at 90^{o}C up to 60 min. Percentage of free radical scavenging ability was taken as a measure to determine the changes in the antioxidant capacity of honeys. Determination of changes in the total polyphenols content (TPC) (equivalents of gallic acid mg/100 g of honey) were also done. The main observation is that compared with conventional process, honey treated with microwave field has faster formation of HMF. Though the effect of a microwave field accelerated greatly

the formation of HMF, but it is suitable for honey processing because of the drastic reduction in operation time.

Maria *et al.* (2014) studied *L. monocytogenes* inactivation kinetics under microwave and conventional thermal processing in kiwi fruit puree. It was revealed that the level of microwave power applied had a considerable influence on the *L. monocytogenes* inactivation rate. The higher the microwave power level, the faster the inactivation. The inactivation of *L. monocytogenes* under microwave heating at 900 W ($D_{60°C}$ = 17.35 s) and 1000 W ($D_{60°C}$ = 17.04 s) happened faster than in a conventional thermal process ($D_{60°C}$ = 37.45 s). Consequently, microwave heating showed greater effectiveness for *L. monocytogenes* inactivation than conventional heating.

Maria *et al.* (2015) performed a comparative study between microwave and conventional heat processing of kiwi fruit puree. In this study, the impact of microwave (1000 W – 340 s) and conventional heat (97^{o}C – 30 s) pasteurization and storage (4, 10, 22^{o}C for up to 63 days) on individual as well as total carotenoids and chlorophylls in kiwifruit puree was evaluated. Studies pertaining to bio accessibility of carotenoids, before and after pasteurization, during storage was also studied. Remarkable changes in carotenoid (62–91 per cent losses) as well as chlorophyll (42–100 per cent losses) contents were observed during conventional and microwave heating. The decrease of total carotenoids and chlorophylls over time, were explained properly by first and second order kinetics respectively. During processing and storage bio accessibility of carotenoids remained ($p < 0.05$) unaltered. These results showed that the pigment composition of microwaved kiwifruit was most likely to that of the fresh fruit and no changes were observed during storage.

Saritha *et al.* (2015) conducted a study on influence of microwave energy on pectic principles of mango peel. The main conclusion was that when used microwave energy for heating purpose, sufficient heat energy was generated within shorter periods of time. Also, where there is a high increase in temperature is desired there microwave

energy can be substituted. Compared with conventional mode of extraction, maximum pectin yield was obtained in a short period of time when microwave energy was used. Better gelling characteristics, high viscosity and methoxyl content of pectin were observed in the samples treated with 660 and 1000 W for 20 min. Maximum yield of pectin was found in the samples exposed to microwave energy of 1000 W for 20 min. If samples exposed to microwave energy for a duration of 25 min, then there is a decrease in methoxyl content, viscosity and galacturonic acid were noticed.

L. monocytogenes is a pathogen of great concern in minimally processed because of its all-over presence and psychrotrophic nature, with a particular ability to multiply at low temperatures, low water activity levels, acidic pH, and also allowing it to reach levels high enough to cause human diseases. In order to achieve or possibly enhance the shelf life of tomato juice, relating to quality and nutritional aspects, microwave heating is considered as one of the appreciable alternative among the novel thermal technologies. Stratakos *et al.* (2015) compared microwave heating and conventional pasteurization by processing tomato juice. The results were displayed in terms of antioxidant activity, microbial load and physicochemical characteristics. No significant changes were observed in physicochemical and colour characteristics of juices were observed during storage. Microorganisms were inactivated and found to be at low levels throughout the storage in both conventional and microwave pasteurization. Increase in cytoprotective effect against H_2O_2 were observed in the juice processed with the microwave energy. Similarity in the two tomato juices was proved by the organoleptic analysis. Hence, the continuous microwave volumetric heating system seems to be a feasible alternative to conventional pasteurization.

2.3.1 Principle of Microwave Heating

Datta *et al.* (2000) stated that, the phenomenon of materials to absorb microwave energy and transform into heat is the main reason for microwave heating of materials. The two important mechanisms by which microwave heating of food materials mainly occurs are ionic polarization and dipolar rotation. Water has dipolar

nature and dielectric heating is due to the presence of water or moisture in a given material. When an oscillating electric field is incident on the food materials, the water molecules which are permanently polarized dipolar molecules try to realign in the direction of the electric field. As the microwave frequency is very high about 2450 MHz, the water molecules vibrate 2450 million times per second which causes internal friction in between the molecules. This friction between the molecules leads to volumetric heating of the material.

Fan *et al.* (2013) concluded that there is no significant difference in the ordered structure of starch granules when heated using microwaves and rapid heating in an oil bath. Slight variations in the proportions of amorphous starch, double helices and V-type single helices, were determined by conventional heating while heating using microwave energy did not have a significant impact on the ordered structures of starch granules.

Bakibaev *et al.* (2015) concluded that the process of getting polylactic acid (PLA) by microwave energy is hundreds of times faster compared with conventional heating. This observation was made when performed an experiment on polymerization of lactic acid using microwave and conventional heating. There is no change in the optical properties of PLA samples obtained in both the processes.

Liu and Lanier (2016) stated that microwave heating results in an increase in palatability of the product especially comminuted meat batters, and the cooking properties were acceptable especially water/fat holding and texture (fracture and small strain mechanical properties). The experiments were performed with meat batters containing high fat content.

2.3.2 Dielectric Properties of Food Material

In order to predict the heating rates and also to know the behavior of various food materials when exposed to high frequency electromagnetic waves in dielectric heating or novel thermal treatments, much interest has been concentrated mainly on

dielectric properties of agricultural materials and food products (Venkatesh and Raghavan, 2004; Sosa-Morales *et al.*, 2010).

Ikediala *et al.* (2000) stated that the dielectric properties of materials can be defined in terms of their relative permittivity. The permittivity related to free space is known as relative complex permittivity (ε_r), and it is represented as:

$$\varepsilon_r = \varepsilon_r' - j\varepsilon_r'' \qquad \text{.....(2.1)}$$

where ε_r' and ε_r'' are known as the dielectric constant and loss factor, respectively. The dielectric constant or real part (ε_r'), tells about the material's ability to absorb microwave energy when it is exposed to electric field. It gives the information about distribution of electrical energy and phase of waves travelling through the medium. The loss factor, or the imaginary part (ε_r''), influences both energy absorption and attenuation, and describes the material's ability to dissipate energy with respect to applied electric field or various polarization mechanisms, which finally leads to heat generation. The amount of heat energy generated in the food is proportional to the value of the loss factor (Tang, 2005).

According to Sosa-Morales *et al*. (2009), penetration depth and electrical conductivity are the other properties related to dielectric parameters. The penetration depth is usually defined as the depth into a sample where the microwave and RF power has dropped to 1/e (e=2.718) or 36.8 per cent of its transmitted value. The penetration depth is a function of ε_r' and ε_r''.

Llave *et al.* (2016) observed the dielectric properties of tylose water pastes during microwave heating and thawing. In this study, good additive for increasing the dielectric loss factor was confirmed to be salt; however increase in thawing time and non-uniformity in heating due to decreased penetration depth were the results of higher salt addition.

2.4 MICROWAVE ASSISTED OIL EXTRACTION

Handa (2008) stated that the traditional methods of producing essential oils are hydro distillation (water distillation, water and steam distillation and direct steam distillation), expression, extraction with cold fat etc. The choice of a particular process for the extraction of essential oil is generally dictated by the following considerations:

a) Sensitivity of the essential oil to the action of heat and water

b) Volatility of the essential oil

c) Water solubility of the essential oil

Steam distillation is not practiced for extracting the essential oils which are highly soluble in water and to those which are likely to decompose when subjected to heat. In order to adopt steam distillation the oil should be steam volatile. All most all the essential oils in commerce are steam volatile, do not decompose when subjected to high heat and practically not soluble in water. Such essential oils are suitable to be processed by steam distillation.

Hydro distillation (Method A) differs from steam distillation (Method B) mainly in that the plant material is almost entirely covered with water in the still which is placed on a furnace. An important factor to consider in hydro distillation is that there must be always enough water present in the tank throughout the distillation process, otherwise the plant material may overheat and char. In this method, water is made to boil and the essential oil is carried over to condenser along with the steam which is formed. Hydro distilled oil is slightly darker in color and has much stronger still notes than oils produced by other methods (Sudeep, 2008).

Though Method A and Method B are most commonly used they possess some disadvantages such as more time consuming for the process of extraction, compounds altering and degradation of compounds that takes place due to high temperatures, low

oil yield and high energy consumption for the complete extraction process (Lucchesi *et al.,* 2004; Chen *et al.,* 2016).

Hong *et al.* (2001) observed the changes in the extraction of phenolic compounds from grape seeds using microwave energy as heating source. In this optimization study microwave power varied from 150 to 300 W and extraction time varied from 20 to 200 s. By using Folin-Ciocalteau reagent the polyphenol content of the final extracts was measured in terms of mg of tannic acid equivalent per gram of crude extract (mg TAE/g of crude extract). The main observation is that, the yield of extract increased to 15.2 per cent and the polyphenol content increased to 429 mg TAE/g of crude extract when the solvent polarity was changed by the addition of 10 per cent water.

Luque-Garcia *et al.* (2002) proposed a new method for extraction of fat from prefried and fried meat and fish. A drastic reduction in the process time (55 min versus 8 h) was achieved with same reproducibility that may be obtained by the conventional method. Besides, the proposed method has the advantage of recycling around 75-80 per cent of the extractant and it is cleaner when compared with conventional Soxhlet.

Chemat *et al.* (2006) studied microwave accelerated steam distillation of essential oil (MASD) from lavender flowers. It was revealed that in steam distillation (SD) and MASD the extraction temperatures were same as the boiling point of water i.e. 100^{o}C at atmospheric pressure. When SD and MASD were compared, the time taken to attain the extraction temperatures and also to get the first essential oil droplet, MASD requires only 5 min when compared with SD which requires almost 30 min. As a result the yield of oil obtained at an extraction time of 10 min in MASD was same as that obtained after 90 min by means of SD, which is one of the advanced methods in the stream of essential oil extraction. The final yield of essential oil obtained from lavender flowers was 8.86 per cent by MASD and 8.75 per cent by SD. The energy required to perform the two extraction methods are 1.5 kWh for SD and 0.13 kWh for MASD, respectively.

Chemat *et al.* (2006) stated that extraction time in microwave assisted process was found to decrease with increase in temperature. This decrease could be attributed to the fact that with increase in temperature, the vapour pressure of water present inside the celery seeds increased leading to leaching out and evaporation of volatile oil along with water.

Lucchesi *et al.* (2007) studied solvent-free microwave extraction (SFME) of cardamom essential oil. The results showed that compared to conventional hydro-distillation, rupture of glands and plant were more rapid when heated with microwave energy. In case of microwave heating, when the glands were subjected to more severe thermal stresses and localized high pressures, pressure build-up takes place within the glands which results in increased capacity for expansion, and leading to cell rupture more rapidly than in conventional extraction. Statistical treatment of the results revealed that the selected parameters i.e. extraction time, irradiation power and moisture content of the seeds have significant effect on the output parameters. The essential oils were analyzed by gas chromatography–mass spectrometry (GC–MS). Essential oils provided by SFME are dominated by the oxygenated fraction which is more valuable and composed of highly odoriferous aromatic compounds.

Golmakani *et al.* (2008) compared the microwave-assisted hydro distillation (MAHD) with the traditional hydro distillation (HD) method in the extraction of essential oils from *Thymus vulgaris* L. The results showed that MAHD has taken only 75 min for extracting essential oil when compared to 4 h in HD. Also, MAHD was superior in terms of saving energy. Scanning electron microscopy (SEM) of thyme leaves undergone HD and MAHD provided evidences that sudden rupture of essential oil glands takes place in MAHD. The refractive indices, specific gravities and colour of essential oils obtained from thyme aerial parts for both MAHD and HD fall within the ranges specified by Food Chemical Codex (FCC). Gas chromatography–mass spectrometry analysis of the extracted essential oils indicated that the use of microwave energy did not adversely affect the composition of the essential oils.

Leslie and Maria (2011) developed a microwave assisted method with the aim of improving the extraction efficiency of Theobromine and Caffeine from cacao. The results showed that the microwave method was more efficient when compared with conventional method and the extraction efficiency increased from 15 per cent to 72 per cent in case of Theobromine and 36 per cent to 153 per cent in case of Caffeine. Also the method was found to be precise, fast and easy.

Desai and Parikh (2012) performed a comparative study on microwave assisted extraction of essential oil from the leaves of *Cymbopogon Flexuosus* (Steud.) Wats. (Lemon grass). The effect of various parameters like solid loading, volume of water, rehydration time, extraction time, and power on yield and composition of essential oil was examined. Better quality was obtained for the essential oil extracted by MAE under the conditions of 20 per cent solid loading, 500 ml water, 1 h rehydration time, 45 min extraction time, and 850 W power. Yield of essential oil was found to be the same (1.04 per cent) for HD and MAE. HD required 90 min to treat 50 g of plant material with an energy consumption of 0.75 kWh while MAE was complete in 45 min by treating 100 g of plant material and using 0.6375 kWh. Thus, with reduced energy consumption and carbon footprints, MAE can be considered as a potential green method for extracting essential oil from the leaves of lemongrass. The essential oils extracted either by MAE or HD has almost similar chemical constituents; however, the percentage varies with respect to the technique employed. Citral is the main component found in essential oil extracted by either technique. A higher amount of citral (80.01 per cent) is present in oil extracted by MAE compared to that by HD (72 per cent).

Kiruba *et al.* (2013) optimized the microwave assisted process for extraction of Phenolic antioxidants from grape seeds (*Vitis vinifera*) which are rich in phytochemicals that have antioxidant properties. The effect of independent variables such as microwave power (100, 150, and 200 W), extraction time (2, 4, and 6 min), and solvent concentration (30 per cent, 45 per cent, and 60 per cent ethanol) on

dependent variables i.e. total phenols, antioxidant activity (1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP)) were determined. Central Composite Design was used to optimize the Microwave-assisted extraction (MAE). Extraction time and solvent concentration were the two independent variables significantly influenced the total phenols that were expressed as gallic acid equivalents (GAE), catechin equivalents (CAT), and tannic acid equivalents (TAE). The optimized conditions were maximized for 6 min of MAE of grape seed with 32.6 per cent ethanol at 121 W with a desirability function of 0.947. Per gram of grape seed the predicted extraction yields were 13±0.89, 21.6±1.59 and 15.9±1.32 mg GAE, CAT, and TAE, respectively. In order to inhibit the DPPH the predicted antioxidant activity per gram of dry weight grape seed was 80.9 per cent and 135 μM ascorbic acid equivalents for FRAP test.

Baron and Villa (2014) studied microwave assisted extraction of essential oil and pectin from orange peel in different stages of maturity. The results mainly highlighted the yield of essential oil and the limonene content of the samples obtained using microwave energy. It revealed that the essential oil yield was slightly higher using additional water under the best extraction conditions (600 W, 10 min), and the limonene content, determined by GC- MS, was between 90.5 and 97.9 per cent. It was noticed that at a low power of 200 W, no essential oil was extracted and at a high power level more than 600 W results in an oil colour of dark yellow or even black due to the presence of suspended material. During microwave irradiation intercellular expansion of plant tissues occurred and it was found using Scanning electron microscopy (SEM) analyses after essential oil extraction. Without the addition of solvent, leads to orange peel carbonization when the microwave power was higher than 600 W. The content of essential oil decreased with the maturity (0.14 to 0.08 per cent).

With increase in microwave power and decrease in solvent the peels begun to carbonize and further increase in time leads to completely charred and black coloured

sample. Also, with increase in solvent the pectin extraction yield also has got enhanced (Kratchanova *et al.*, 2004; Baron and Villa, 2014).

Gopika and Ghuman (2014) developed a microwave assisted extraction unit for extraction of essential oil from celery seeds. The extraction unit was developed by modifying a domestic microwave oven and attaching a Clevenger apparatus to extract the essential oil. Effect of various independent variables such as soaking time, temperature and power density on celery seed during MAE was noticed. Box-Behnken design which is a multivariate study was used to evaluate the influence of the process parameters on the performance of MAE on celery seed. A comparative study was made with the oil yield, extraction time and energy consumption ($MJ.kg^{-1}$ oil) obtained by MAE with those of traditional hydro-distillation (HD). Results revealed that microwave assisted process gave approximately same oil yield (1.90 per cent) in less time (93.5 min) and with low energy consumption (58191.78 $MJ.kg^{-1}$ oil). It implies the selected parameters had significant effect on the responses.

Also, the results revealed that the lower yield of oil extracted at 90^{o}C might be due to the temperature being not enough to burst open the oil glands. Oil yield was also lower at 110^{o}C because evaporation rate was higher than the condensation rate. Also, soaking time was found to have significant effect on oil yield. Increase in soaking time, leads to increase in pressure inside the seeds till bursting of outer layer took place. This bursting led to release of oil, which increased the oil yield when compared to conventional hydro-distillation process. With increase in soaking time, oil yield decreased to a point of minima at 8 h. With further increase in soaking time, oil yield increased, but to a lesser value than at 4 h.

Avelina *et al.* (2016) studied the effect of different process parameters during microwave assisted extraction (MAE) on the yield of essential oil obtained from orange peel. There was a significant effect ($p < 0.05$) of the particle size, moisture content and its interaction on the essential oil yield obtained and had an influence on the extraction phenomenon. The yield of oil during microwave assisted process is more by 0.9 per

cent than oil obtained by hydro distillation process. Besides, the process reduce the processing times. Decrease in particle size, increases the superficial area which provides a better contact of the sample with the solvent and penetration of microwaves and improves the extraction process.

High moisture content enhances the extraction recovery in most cases, due to rapid heating and temperature increase. This is because of microwaves interacting selectively with the free water molecules present in the gland and vascular system, leading to rupture of walls and release of the essential oil into the solvent (Letellier and Budzinski, 1999; Avelina *et al.*, 2016;). But Ferhat *et al.* (2009) stated that during lavender flowers essential oil extraction by microwave, there was no much difference in the yield of essential oil obtained by steam diffusion but the process time has got reduced drastically.

2.5 COMBINATION TECHNOLOGIES

Though there are many advantages with the microwave technology like reduction of process times, energy consumption etc. but the major problem was localized heat zones related with the variation in physical, dielectric and thermal properties of food components. In order to decrease the localized heat zones in foods, microwave heating assisted with conventional heating methods such as vacuum and microwave absorbents was advantageous.

You *et al.* (2007) performed an experiment to determine triazines in infant nutrient cereal-based foods by pressurized microwave-assisted extraction (PMAE) coupled with high-performance liquid chromatography and mass spectrometry. The recoveries increased from 66.2 to 88.6 per cent by using PMAE. Compared with atmospheric pressure microwave-assisted extraction (AMAE), ultrasonic extraction (UE) and soxhlet extraction (SE), the proposed method was more efficient, faster and more straightforward and required no additional cleanup steps. When the proposed method was applied to the aged spiked nutrient cereal samples, the results indicated

that, although the recoveries of analytes were much lower than those obtained from fresh spiked samples, they were nevertheless satisfactory for the quantitative analysis of practical samples. The highest recoveries were obtained in the time ranging from 8 to 10 min, while low recoveries were obtained when the extraction time is shorter than 8 min and longer than 10 min. On the other hand, the low recoveries at short irradiation time might be due to insufficient microwave energy, which can be available to attain the temperature of phase change and hence enable the breaking of the analyte–matrix bonds or might result from the strong adsorption of the analytes on the sample particle surface. Also long extraction times can cause degradation of the thermo liable compounds.

Nguyen *et al.* (2013) designed and fabricated a continuous flow simultaneous microwave and ohmic combination heater to heat treat particulate foods without leaving solids under processed. The results showed that maximum solid-liquid temperature differences under microwave and ohmic heating were about 8.1 and 8.0^{o}C, respectively. However, when microwave and ohmic heating techniques were applied simultaneously, there was no significant temperature difference between solid and liquid phases. Energy efficiency of combination heating was higher than microwave heating and a maximum increase in energy conversion of 12.8 per cent was obtained.

Lee *et al.* (2015) developed a dual cylindrical microwave and ohmic combination heater for minimization of thermal lags in the processing of particulate foods. Results showed that two parameters in ohmic heating mainly salt concentration and particle size affected temperature variations between solution and particulates. Irrespective of particle size and mass fraction, the solution temperature was less than the particle temperature in microwave heating up to 12.5 g/l salt concentrations. However, if salt concentration in food mixtures increased to 20 g/l an opposite tendency was observed.

Samani *et al.* (2015) analyzed the combinative effect of ultrasound and microwave power on *Saccharomyces cerevisiae* in orange juice processing. Due to

overheating of juice at the heat-exchange surface in conventional heat pasteurization of orange juice off flavour in juice was detected. Taste of the juice is not changed when heated using combination technology. Also, complete inactivation of bacteria and pectin methyl esterase was obtained in the combined process. There was no significant effect on juice flavour. Also the appearance of orange juice in the combinative method was better than those of conventional method (57 per cent vs. 43 per cent).

Chen *et al.* (2016) studied a two stage microwave extraction of essential oil and pectin from pomelo peels and stated that microwave can enhance the extraction process by two distinct mechanisms: one attributes to the diffusion across the intact oil gland while the other involves the convection through the broken oil gland. Usage of extreme extraction condition, especially high temperature leads to instability of essential oil i.e. bringing negative effects such as thermal degradation of essential oil. Also when microwave power is low prolonging extraction time would be helpful to complete extraction of target compounds. The percentage of limonene increased with increasing microwave power at low microwave powers of 150 and 300 W, but decreased in high microwave power of 450 W. The findings suggested that microwave extraction at low microwave power may be suggested as an effective technique for the extraction of essential oils because of its higher yield and better quality of essential oils when compared with hydro distillation (HD).

Fangyuan *et al.* (2016) studied cyclodextrin based ultrasonic assisted microwave extraction. They concluded that the presence of cyclodextrin or ethanol significantly increased the extraction efficiency of the analytes. Secondly, ultrasound assisted microwave extraction provided the highest extraction yields demonstrating that ultrasound and microwave are crucial parameters in the extraction efficiency. UAME extracts compounds from herbal matrices in very short periods of time through the synergistic effect of acoustic effects and microwave radiation.

From the review of literature it is understood that microwave energy could be effectively used for extraction of essential oil in place of steam or water heating. The

main advantage of using microwave energy is it significantly increases the speed of the processes and reduces the thermal gradients. The essential oil from many plant materials were successfully extracted using microwave energy, but the studies pertaining to nutmeg mace essential oil extraction using microwave energy has not been found reported. With this background, the present study envisages development of a system for microwave extraction of nutmeg mace oil and optimization of the process parameters leading to high extraction efficiency and quality of the extracted oil. Such a study could produce nutmeg oil of high quality and quantity and reduce operational costs.

CHAPTER III

Materials and Methods

This chapter describes the conceptual design and development of a microwave assisted process for extraction of nutmeg mace essential oil. The materials used for fabrication of the various components and the instrumentation employed for measurement of parameters were explained. The process of evaluation and optimization of process parameters for extraction of mace essential oil with maximum oil yield, minimum time and energy consumption and the methods for determining the physical and chemical properties of essential oil were detailed.

3.1 DEVELOPMENT OF MICROWAVE ASSISTED UNIT FOR EXTRACTION OF OIL

Based on a thorough review of works carried out on microwave assisted oil extraction, the design of a small capacity oil extraction unit assisted by microwave was conceptualized, further refined and then fabricated. The developed experimental system as shown in Fig 3.1 and Plate 3.1 consists of the following main components:

1. Microwave cavity
2. Extraction unit
3. Supporting stand
4. Energy meter

3.1.1 Microwave Cavity

The prime requirement for the microwave assisted extraction process is a microwave source. Commercially available microwave ovens could be effectively utilized for this purpose. The selection of microwave oven should be based on the power consumption. For laboratory scale experiments, ovens with maximum power delivery of 1000 W was generally choosen (Chemat *et al.,* 2006; Lucchesi *et al.,* 2007;

Jiao *et al.*, 2012). Accordingly, a microwave oven with (Model: Magicook MW20BC) with following specifications was used to serve as the microwave source.

Power consumption	230 V/50 Hz, 1200 W (Microwave)
	1100 W (Grill)
	2000 W (Convection)
Operation frequency	2450 MHz
Outside dimensions	262 mm(h) x 452 mm(w) x 395 mm(D)
Oven cavity dimensions	195 mm(h) x 315 mm(w) x 325 mm(D)
Oven capacity	20 litres
Cooking uniformity	Turntable system

The oven consists of a control panel where cooking time, power, action indicators and clock time are displayed and controlled. The oil is extracted by micro mode since the temperatures are low at this mode. The power could be increased in steps of 10's such as P-70, P-80, P-100 etc. in which P-100 indicates that oven utilizes 100 per cent of its rated power i.e. 1200 W for extraction of oil. The time for extracting the oil can be set by pressing the time button on the control panel.

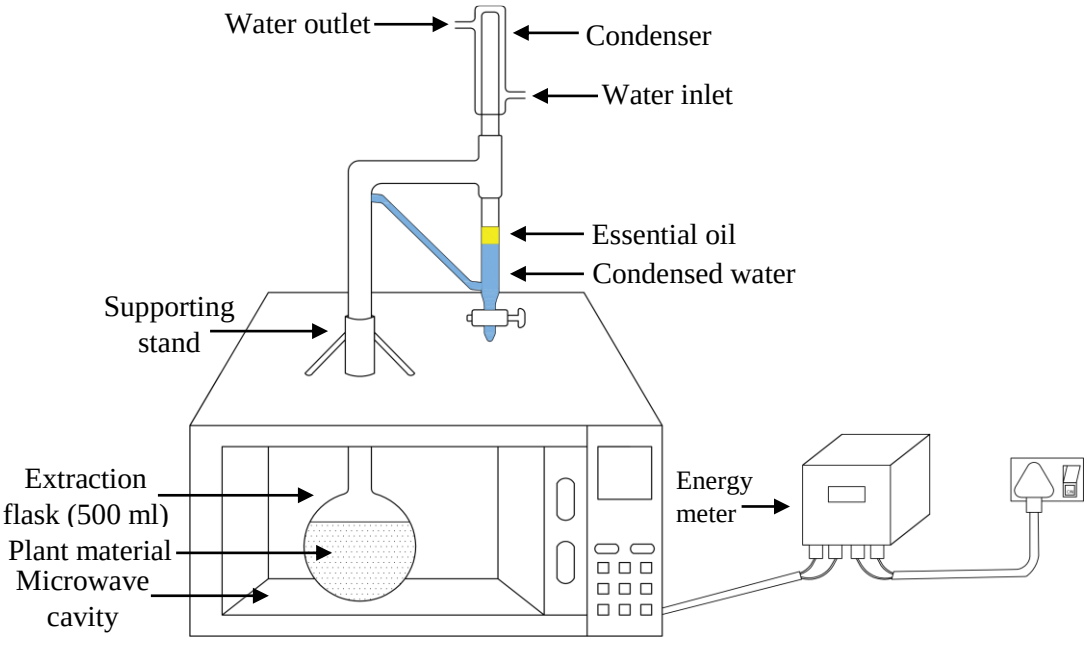


Figure 3.1. Schematic of the microwave assisted extraction unit

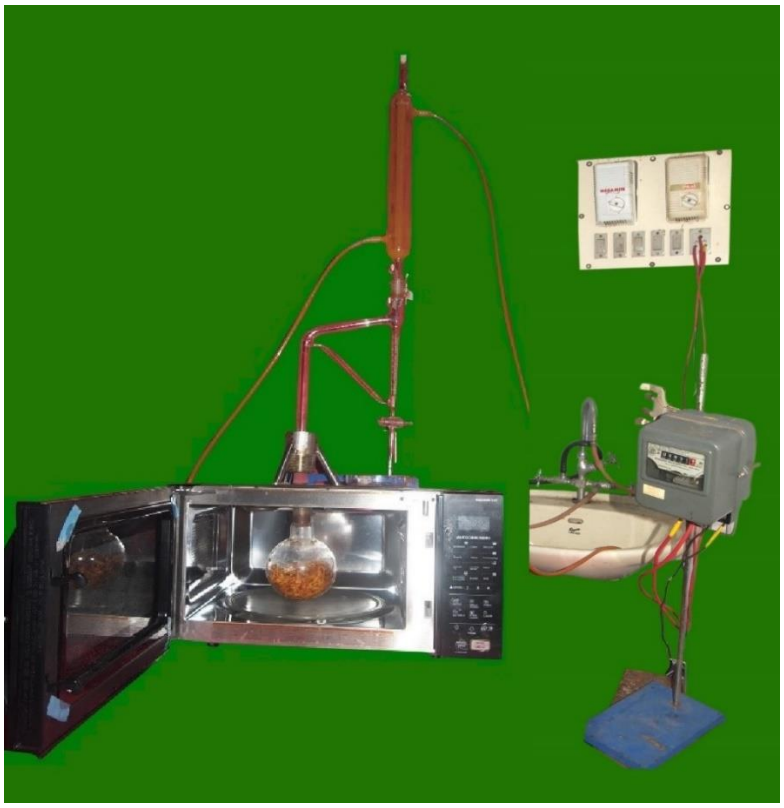


Plate 3.1. Developed microwave assisted extraction unit

3.1.2 Extraction Unit

The Extraction unit consists of a Clevenger hydro distillation system in which recycling of distilled water also takes place. Clevenger hydro distillation system consists of a round bottomed flask, Clevenger and a condenser (Plate 3.2). The capacity of the round bottom flask match with the dimension of the microwave cavity because of its ease in inserting and removing the flask while loading and unloading the plant material (Chen *et al.*, 2016). A hole of ½ inch diameter was drilled on the top of the microwave cavity in order to fix the condenser into the round bottom flask placed in the cavity. The round bottomed flask and the condenser was connected by means of a glass tube (1/2 inch diameter) and two rubber corks. Half portion of glass tube is projected upwards and remaining half portion is inside the oven cavity holding the

round bottomed flask. The glass tube acts as carrier of vapors, both water and essential oil.

3.1.3 Supporting Stand

The supporting stand is fabricated by using stainless steel material. It is placed outside the oven for supporting the glass extraction unit. The stand comprises of a circular ring and three stainless steel pipes which acts as supporting legs for the ring. The circular ring has an outer diameter of 48 mm and inner diameter of 43 mm. The height of the circular ring is 69 mm. The three pipes each of diameter 12.7 mm and length 80 mm is welded to the circular ring. For keeping the distillation unit straight without tilting a rubber cork was inserted into the circular ring through which the glass stem passes. The inner and outer diameters of the cork are 23 mm and 43 mm, respectively with a cork length of 20 mm. The supporting stand is shown in (Plate 3.3).

3.1.4 Energy Meter

A single phase induction type energy meter (Plate 3.4) was connected to the microwave assisted extraction system to measure the energy consumed during the distillation process. The energy consumed for microwave assisted extraction process at different process levels as per the experimental design and for conventional hydro distillation process were measured for comparison of the energy efficiency of the microwave assisted process.

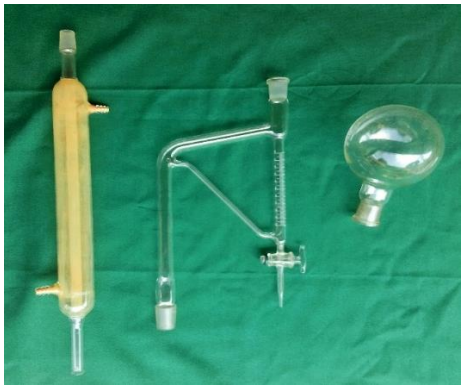


Plate 3.2. Components of the extraction system

Plate 3.3. Supporting stand

Plate 3.4. Energy meter

3.2 PHYSICAL QUALITY CHARACTERISTICS OF OIL

3.2.1 Refractive Index

The refractive index of a transparent liquid was determined by the method of reflected system of Newton's rings for which Newton's rings apparatus, Sodium vapour lamp, and Vernier microscope were used (Plate 3.5). The Newton's rings apparatus consists of an optically plane glass plate P on which is placed a convex lens L of large focal length. Above the lens, another glass plate G is arranged at 45^0 to the horizontal. The complete set up is shown in Plate 3.6. When the lens is placed over the glass plate, the space between the lens and glass plate contains air.

If D_m and D_{m+k} be the diameters of the m^{th} and $(m+k)^{th}$ dark rings respectively, Then,

$$D_m^2 = 4mR\lambda$$

$$D_{m+k}^2 = 4(m+k)\,R\lambda$$

$$D_{m+k}^2 - D_m^2 = 4kR\lambda$$

Where λ is the wavelength of the light used and R is the radius of curvature of the lens. With a thin film of the transparent liquid between the lens and the glass plate, if D_m'

and D_{m+k}' are the diameters of the m^{th} and $m+k^{th}$ rings, $D_{m+k}'^2 - D_m'^2 = 4KR\lambda/n$ where n = refractive index of the liquid.

From the two equations,

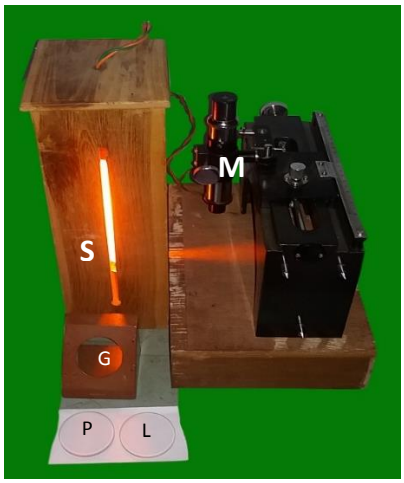
$$n = D_{m+k}^2 - D_m^2 / D_{m+k}'^2 - D_m'^2 \quad \ldots. (3.1)$$

Light from a sodium vapour lamp S is rendered parallel by a short focus convex lens. The parallel rays fall on the glass plate G, inclined at 45° to the horizontal, gets reflected and then fall normally on the convex lens L placed over the glass plate P. A system of bright and dark concentric circular rings are observed through a microscope M, arranged vertically above the glass plate G as shown in Plate 3.7. The microscope is properly focused so that alternate bright and dark circular the rings are seen clearly.

By working its fine adjustment screw of the microscope make sure that there are about 25 clear rings on either side of the centre. Starting from the centre of the fringe system, the microscope is moved towards the left so that the cross-wire is tangential to the m^{th} (say 20^{th}) dark ring. The microscope reading on the horizontal scale was taken. By working the fine adjustment screw, the microscope was moved towards the right. The cross-wire was adjusted to be tangential to the 18^{th}, 16^{th} etc. dark ring is succession up to the second dark ring on the left and the corresponding readings were taken. Then the cross-wire was made tangential to the second dark ring on the right side. Readings were taken corresponding to the 2^{nd}, 4^{th} …. 20^{th} dark ring, as before. The difference between the readings on the left and right of each ring gives the diameter D of the respective ring. Hence $(D_{m+k}^2 - D_m^2)$ was calculated.

A drop of liquid is placed on the plane glass plate and the lens was placed over it. The lens and the glass plate were pressed together so that a thin film of liquid without any air bubble is formed between them. The experiment was repeated as before and D_m' and D_{m+k}' were measured. The refractive index of the liquid was then calculated.

Refractive index (n) = Mean of $(D_{m+k}^2 - D_m^2)$ / Mean of $(D_{m+k}'^2 - D_m'^2)$ …. (3.2)



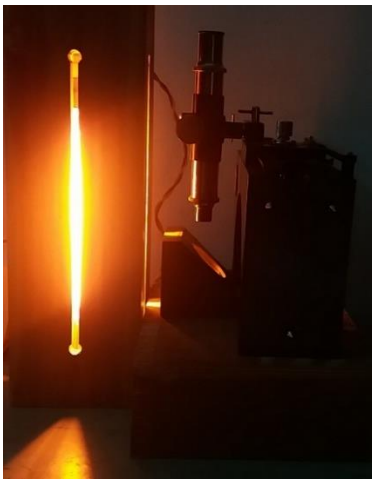


Plate 3.5. Newton's rings apparatus Plate 3.6. Refractive index experimental set up

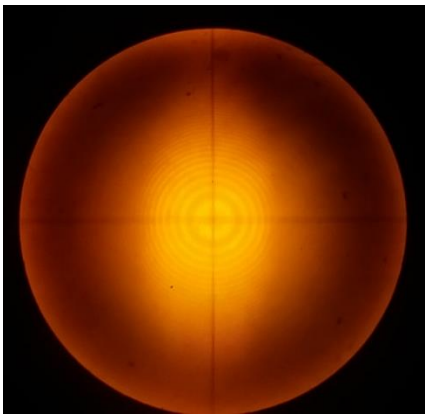


Plate 3.7. Circular rings

3.2.2 Specific Gravity

Specific gravity was measured by dividing the weight of 1 ml essential oil by the weight of 1 ml distilled water. Weights were calculated using a balance with an accuracy of 0.001g (Gopika and Ghuman, 2014).

3.2.3 Solubility Test

The solubility of nutmeg essential oil was determined based on the procedure suggested by Food Chemical Codex (FCC, 1996). One ml sample of nutmeg essential oil was transferred into a calibrated 10 ml glass stoppered cylinder graduated in 0.1 ml

divisions. The oil was then diluted with 0.1 ml of 85 per cent (v/v) ethanol repeatedly each time. The temperature was maintained at 25^{o}C, and the contents mixed thoroughly after each addition of alcohol. The dilution procedure was continued until a clear mixture was obtained. The volume of alcohol (V) used to obtain a completely clear solution was recorded. Once the clear solution was obtained, the dilution process was continued, but with 0.5 ml 85 per cent ethanol until the volume of alcohol added was 20 times the volume added earlier. The solution was thoroughly shaken each time with 0.5 ml ethanol until no turbidity was observed. The results were expressed as ‘‘one volume of essential oil soluble in V volumes or more of 85 per cent ethanol”.

3.2.4 Colour

The colour of the Nutmeg mace oil was found using a Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virgina, USA; model: HunterLab’s ColourFlex EZ) (Plate 3.8). The Hunter lab’s colour flex spectro calorimeter consists of measurement (sample) port, opaque cover and display unit. This colour flex meter works on the theory of focusing the light and measuring energy reflected from the sample across the entire visible spectrum. For matching a sequence of colour across the visible spectrum, primary lights are required and describes the colour by mathematical model called as Hunter model. It reads the colour of sample in terms of L, a and b values where, luminance (L) forms the vertical axis, which indicates whiteness to darkness. Chromatic portion of the solids is defined by: a (+) redness, a (-) greenness, b (+) yellowness, and b (-) blueness.

A transparent glass cup filled with sample was placed over the port of the instrument and an opaque cover which act as a light trap to exclude the interference of external light was placed over the cup. Before actual measurements colour was calibrated by fixing the definite colours like white and black tiles. After calibration, the sample was placed over the port and values of ‘L’, ‘a’ and ‘b’ were recorded.

Plate 3.8. Hunter Lab colourimeter

3.3 EXPERIMENTAL DESIGN

Based on a thorough review of literature and the preliminary studies conducted, the process parameters which would influence the essential oil yield, extraction time and energy consumption were chosen as independent variables. The physical quality characteristics which are characteristics of these parameters were selected as dependent variables.

3.3.1 Independent Variables

a) Solid : Water ratio (S):

1) S_1 : 1:14

2) S_2 : 1:10

3) S_3 : 1:6

b) Microwave power density (P):

1) P_1 : 9.6 $W.g^{-1}$

2) P_2 : 14.4 $W.g^{-1}$

3) P_3 : 19.2 $W.g^{-1}$

c) Soaking time (T):

1) T_1 : 2 h

2) T_2 : 3 h

3) T_3 : 4 h

3.3.2 Dependent Variables

Microwave assisted extraction system output parameters:

a) Essential oil yield
b) Extraction time
c) Energy consumption

Physical quality characteristics of nutmeg essential oil:

a) Colour
b) Refractive index
c) Specific gravity
d) Solubility

3.4 STATISTICAL ANALYSIS

Unlike conventional empirical optimization, the statistical optimization method can take into account the interaction of variables in generating the process response. Response Surface Methodology (RSM) was chosen in the experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables and the objective is to optimize this response (Montgomery, 2001). The main advantage of RSM is to reduce the number of experimental runs needed to provide sufficient information for statistically acceptable results. Three variables i.e. solid: water ratio, power density and soaking time at three levels were chosen based on preliminary trials conducted prior to final

experimentation. A Box–Behnken design of three variables and three levels, each with three centre point combinations, was used (Box and Behnken, 1960). This design was adopted as it fulfilled most of the requirements needed for optimization of the microwave assisted process. In the above design the three levels of the process variables were coded as -1, 0, +1 and designed in coded X form (Gopika and Ghuman, 2014). The values of independent variables at three levels were shown in Table 3.1.

Table 3.1. Values of independent variables at three levels of Box–Behnken design

Independent variable	**Symbol**		**Level**	
	Coded	**Un-coded**	**Coded**	**Un-coded**
Solid : water ratio	X_1	S	-1	1 : 14
			0	1 : 10
			1	1 : 6
Power density ($W.g^{-1}$)	X_2	P	-1	9.6
			0	14.4
			1	19.2
Soaking time (h)	X_3	T	-1	2
			0	3
			1	4

According to Box-Behnken design for three independent factors, the total experiments to be conducted are found to be seventeen. Seventeen experiments were performed with three variables and three levels of each variable as shown in Table 3.2.

Table 3.2. Experimental design used for extraction of MAE of nutmeg mace essential oil

Standard order	Run	Coded variables			Un-coded variables		
		Solid:Water ratio	Power density ($W.g^{-1}$)	Soaking time (h)	Solid: Water ratio	Power density ($W.g^{-1}$)	Soaking time (h)
1	4	-1	-1	0	1 : 14	9.6	3
2	5	1	-1	0	1 : 6	9.6	3
3	14	-1	1	0	1 : 14	19.2	3
4	7	1	1	0	1 : 6	19.2	3
5	12	-1	0	-1	1 : 14	14.4	2
6	17	1	0	-1	1 : 6	14.4	2
7	8	-1	0	1	1 : 14	14.4	4
8	10	1	0	1	1 : 6	14.4	4
9	1	0	-1	-1	1 : 10	9.6	2
10	2	0	1	-1	1 : 10	19.2	2
11	15	0	-1	1	1 : 10	9.6	4
12	13	0	1	1	1 : 10	19.2	4
13	6	0	0	0	1 : 10	14.4	3
14	9	0	0	0	1 : 10	14.4	3
15	3	0	0	0	1 : 10	14.4	3
16	11	0	0	0	1 : 10	14.4	3
17	16	0	0	0	1 : 10	14.4	3

For optimization of the independent variables and to check the adequacy of the experimental design, the second order non-linear regression equation (eq. 3.3) was fitted between dependent and independent variables (Lee *et al.*, 2006).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad \ldots(3.3)$$

Where,

Y is the response variable

b_0, b_1, b_2 and b_3 are regression coefficients of linear terms

b_{11}, b_{22} and b_{33} are regression coefficients of quadratic terms

b_{12}, b_{13} and b_{23} are regression coefficients of cross-product terms

X_1, X_2, and X_3 are the coded values of the independent variables X, i.e. Solid: Water ratio (X_1), Power density (X_2) and Soaking time (X_3), respectively. The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by F-test. The significance of the regression coefficient was determined by p-value. The coefficients of the equation were determined by employing Design Expert Version 9.0 a (Stat-Ease, Inc. USA). Analysis of variance (ANOVA) for the final predictive equation was carried out using Design Expert Software. The response surface equation was optimized for the response variables using the above software. The response surface and contour plot analysis were performed by fixing one independent variable at the middle level while changing the other two.

3.5 EXPERIMENTAL PROCEDURE

In order to evaluate the developed system towards extraction of essential oil, nutmeg mace, which is used as a flavour ingredient in food products possessing high medicinal and therapeutic value and widely grown in Kerala was selected. Fresh nutmeg mace of common variety, collected from local farmer of Tavanur area in Malappuram district of Kerala was used for the study. The detailed procedure for extraction of nutmeg mace essential oil employing the microwave assisted process is detailed below.

3.5.1 Determination of Moisture Content

Moisture content of nutmeg is determined by Dean and Stark Toluene Distillation method as per AOAC (2000).

3.5.2 Extraction of Essential Oil

The desirable amount of nutmeg mace i.e. 50 g is soaked for a various period of time as per the experimental design and the excess water is drained off. The soaked nutmeg sample is shown in (Plate 3.10). The soaked sample is filled in round bottomed flask of the extraction unit. The microwave power level along with time is set in the control panel for various treatment conditions and the set up was switched on. The set power level, the microwaves heat the plant material up to set running time. During this process the vapors of water as well as essential oil in mace gets vaporized and passes out of the microwave cavity through the distillation stem into the condenser. These vapors then passed through the condenser where they gets condensed and falls back into the bottom of the extraction unit where the oil and water gets separated and oil which is lighter than water settles on the top and water which is denser settles on the bottom. After completion of process the oil is collected by means of a stopper provided on the extraction unit. The water is drained off and the essential oil thus collected is dehydrated with anhydrous sodium sulphate and stored at 2^{o}C in amber coloured glass bottles (Plate 3.11) for further analysis.

Conventional method of extraction i.e. Hydro distillation was performed using Clevenger apparatus as control for comparing the microwave assisted process. In the round bottomed flask of Clevenger apparatus, 50 g of sample and 500 ml of distilled water was taken and the whole set up including (Clevenger tube and condenser) was placed on a heating mantle (Desai and Parikh, 2012). The temperature of the heating mantle was maintained at 100^{o}C. The experiment was performed until complete extraction of essential oil from plant material is obtained.

Plate 3.9. Dean and Stark apparatus

Plate 3.10. Soaked nutmeg sample

Plate 3.11. Amber colored glass bottles

3.6 DETERMINATION OF PHYSICAL QUALITY CHARACTERISTICS OF MACE ESSENTIAL OIL

3.6.1 Refractive Index

The refractive index of the mace essential oil was determined using reflected system of Newton's rings (Plate 3.6) as explained in section 3.2.1.

3.6.2 Specific Gravity

The specific gravity of the mace essential oil was determined using the procedure explained in section 3.2.2.

3.6.3 Solubility

The solubility of the mace essential oil in 85 per cent (v/v) ethanol was determined using the procedure explained in section 3.2.3.

3.6.4 Colour

The colour of mace essential oil was measured using Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virgina, USA) (Plate 3.8). Colour of the sample was obtained by measuring 'L', 'a' and 'b' colour values. Essential oil was filled in the colour measuring port of the flex meter up to the mark provided on the colour port and 'L', 'a', 'b' colour values were recorded.

3.7 DETERMINATION OF CHEMICAL CONSTITUENTS

The main aromatic constituent of the nutmeg oil is myristicin. The presence of myristicin and its quantification is taken as a parameter for determining the quality of the extracted oil in international market. In this study the myristicin content of the essential oil extracted through microwave assisted process and hydro distillation process were determined using Gas chromatography (Shimadzu GC-17A, Japan) (Plate 3.12) with a column (30 m in length x 0.25 mm inner dia. x 0.25 µm film thickness), a flame injection detector with an operating temperature of 280^{o}C and an injector with a temperature of 250^{o}C, manual injection and nitrogen as gas carrier. The maximum temperature that can be attained in the equipment was 350^{o}C. The procedure for finding the myristicin content was adopted from Essam and Maytham (2012) and Ester *et al.* (2013). Myristicin standard was obtained from M/s.Sigma, St. Louis, MO, USA.

The standard solution was first injected and the chromatograph of the standard was obtained. Then the sample was injected and its chromatograph was recorded

following the same procedure. The injection was made with an initial split ratio of 1:30 with the injection port temperature of 250^{o}C. The initial temperature was set to 100^{o}C with an initial 1.0 min hold followed by programmed temperature (increment) at the rate of 15^{o}C/min to 60^{o}C followed by holding at the rate of 60^{o}C/ 5 min to 280^{o}C. The chromatographs were then analyzed for myristicin content.

Plate 3.12. Gas chromatograph

CHAPTER IV
RESULTS AND DISCUSSION

This chapter outlines the results on development of a microwave assisted extraction system and evaluation of developed system towards extraction of nutmeg mace essential oil. The outcomes of the procedures laid out for the evaluation leading to the standardization of the main process parameters are discussed in detail. Also, the effect of various process variables on the physical and chemical quality characteristics of the extracted essential oil through microwave assisted process are analyzed, discussed and compared with conventional extraction process.

4.1 DETERMINATION OF MOISTURE CONTENT

The moisture content of the fresh nutmeg mace which was collected from local farmer was determined by using Dean and Stark distillation method as explained in section 3.5.1. The average moisture content of nutmeg mace was found to be 9 per cent (wb).

4.2 DEVELOPMENT OF A MICROWAVE ASSISTED EXTRACTION SYSTEM

A microwave assisted extraction system for extracting nutmeg mace essential oil was developed which consists of microwave cavity, extraction unit, supporting stand and energy meter as shown in Fig. 3.1. The microwave source chosen delivers a maximum microwave power of 1200 W and the oil is extracted by micro mode since the temperatures are low at this mode. The extraction unit mainly comprises of Clevenger hydro distillation system. For supporting this glass extraction system a supporting stand was fabricated and the energy consumed for extracting the nutmeg mace essential oil in hydro distillation (HD) and microwave assisted extraction (MAE) was measured using a single phase induction type energy meter. The plant materials respond differently to the action of microwaves because the heating is through kinetic effects and is a volumetric process. Therefore, the process parameters needs to be standardized.

4.3 STANDARDIZATION OF THE PROCESS PARAMETERS OF THE MICROWAVE ASSISTED EXTRACTION SYSTEM

In order to evaluate the developed system towards extraction of nutmeg mace essential oil and for optimization of the process parameters, a series of experiments with solid: water ratios of 1:14, 1:10 and 1:6, power densities 9.6, 14.4 and 19.2 $W.g^{-1}$ and soaking times 2 h, 3 h and 4 h as input variables were performed. The experiments were performed as per the experimental procedure laid out in section 3.5. The results of the experiments conducted towards the microwave assisted extraction process with mean values of extraction time, oil yield and energy consumption are tabulated in Table 4.2.

Table 4.1. Effect of process variables towards extraction of nutmeg mace essential oil

Sl. No.	Sample	Extraction time (h)	Oil yield (ml)	Energy consumption (kWh)
1.	S_1 P_1 T_1	4.2	4	1.54
2.	S_1 P_1 T_2	3	4.3	1.09
3.	S_1 P_1 T_3	3.4	4.5	1.26
4.	S_1 P_2 T_1	4.6	5.6	1.67
5.	S_1 P_2 T_2	3.3	5.3	1.40
6.	S_1 P_2 T_3	3	5.9	1.09
7.	S_1 P_3 T_1	3.5	4.8	1.49
8.	S_1 P_3 T_2	3	5.3	1.09
9.	S_1 P_3 T_3	2.8	4.6	1.19
10.	S_2 P_1 T_1	4.6	4	1.67
11.	S_2 P_1 T_2	4.1	4.5	1.30
12.	S_2 P_1 T_3	3.8	4.8	1.38
13.	S_2 P_2 T_1	4.3	5.0	1.55
14.	S_2 P_2 T_2	4.7	5.3	1.70
15.	S_2 P_2 T_3	4.1	4.7	1.49
16.	S_2 P_3 T_1	4.9	5.4	1.77
17.	S_2 P_3 T_2	4.3	5.8	1.42

18.	S_2 P_3 T_3	3.5	4.9	1.27
19.	S_3 P_1 T_1	4.6	3.8	1.79
20.	S_3 P_1 T_2	3.1	4	1.12
21.	S_3 P_1 T_3	3.6	4.1	1.40
22.	S_3 P_2 T_1	4.3	5.1	1.56
23.	S_3 P_2 T_2	4.0	4.8	1.47
24.	S_3 P_2 T_3	3.5	4.9	1.27
25.	S_3 P_3 T_1	3.7	4.5	1.36
26.	S_3 P_3 T_2	3.2	4.3	1.16
27.	S_3 P_3 T_3	3.1	3.9	1.19
28.	HD	8	5.8	2.43

For optimizing the parameters, the results obtained in Table 4.2 were used as responses and listed as per the order mentioned in design, as explained in section 3.4. Only seventeen experimental data were used in the design to optimize the parameters as per response surface methodology. In order to relate the independent process variables, a second order quadratic model was used. In the second order polynomial equation, the coefficient of each term was determined using an analysis in Design Expert software named as multiple regression analysis. Regression coefficients were obtained when experimental data was fitted to the selected models. Analysis of variance (ANOVA) helps in examining each response for statistical significance of the terms in the regression equation. Thus ANOVA can determine the adequacy and significance of the quadratic model. To check the significance of each of the coefficients 'p' values were used as a tool. Also, to understand the mutual interactions between test variables 'p' values are necessary. The corresponding coefficient is more significant when 'p' values are smaller in magnitude i.e. ($p < 0.05$). The adequacy of regression model was checked by R^2, Adjusted R^2, Adequate Precision and Fisher's F-test (Montgomery, 2001).

Adjusted R^2 is a measure of the amount of variation around the mean, adjusted for the number of terms in the model. As the number of terms in the model increases,

the adjusted R^2 decreases if those additional terms do not add value to the model. Adequate precision compares the range of predicted values at design points to the average prediction error.

The significance of all terms in the polynomial was judged statistically by computing the F-value at probability (p) of 0.1 to 0.01. A complete second order quadratic model was employed to fit the data and adequacy of the model was tested by considering R^2, Adjusted R^2, predicted R^2 (a measure of how good the model predicts a response value) and Fischer F-test (Haber and Runyon, 1977). During the explanation of variation in behavior the smaller the value of R^2, the less importance the dependent variables in the model have. Partial differentiation of the process parameters was done to optimization the model with respect to each parameter. The resulting function is solved by equating the equation to zero. Statistical calculation was done using the regression coefficients to generate three-dimensional plots for the regression model.

4.4 OUTPUT CHARACTERISTICS OF MAE SYSTEM

4.4.1 Extraction Time

The time taken for extracting nutmeg mace essential oil during various combinations of process parameters are presented in Table 4.2. The extraction time varied between 3 to 4 h 36 min. The minimum time for extracting maximum amount of nutmeg mace essential oil was obtained when the solid: water ratio is 1: 14, soaking time of 4 h and power density of 14.4 $W.g^{-1}$.

Response surface methodology was used to enquire the relationship between the independent and dependent variables. The ANOVA table for the response "Extraction time" is given in Appendix A (Table A.1). The second order non-linear regression equation was fitted between dependent and independent variables using the experimental values. Following regression model was obtained to predict the total time of extraction of nutmeg mace essential oil.

Extraction time = 4.76 +0.063A +0.013B -0.58C +0.025AB +0.20AC -0.15BC

$$-1.02A^2 -0.67B^2 +0.11C^2 \quad \text{.....(4.1)}$$

Where A = solid: water ratio

B = power density ($W.g^{-1}$)

C = soaking time (h)

From Table A.1, it can be concluded that the values of R^2, R^2-adj and R^2-pred for the total time of extraction were 98.35, 96.22 and 96.29 per cent, respectively. The coefficient of determination (R^2) of the regression model for total time of extraction was 98.35 per cent which implies that the model could account 98.35 per cent variability in data. The R^2-pred (96.29 per cent) is in reasonable agreement with the R^2-adj (96.22 per cent). Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (16.73) value for total time of extraction indicates that the model can be used to predict the response within the design space as it is greater than 4.0 (Montgomery, 2001). Therefore, second order model was adequate in describing the total time of extraction of MAE essential oil.

It is evident from the Equation (4.1) that the total time of extraction was in positive correlation with solid: water ratio, power density and in negative correlation with soaking time. It indicates that with increase in soaking time above 3 h, the total time of extraction was found to decrease. Table A.1 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on total time of extraction at 1 per cent ($p<0.001$) level of significance as F- calculated value (46.25) was found greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.4.2 Oil Yield

The yield of nutmeg mace essential oil obtained in various combinations of experiments are shown in Table 4.2. The total yield of oil varied from 3.8 to 5.9 ml. The maximum oil yield was obtained for a solid: water ratio of 1: 14, power density of 14.4 $W.g^{-1}$ and soaking time of 4 h.

The ANOVA table for the response "oil yield" is shown in Appendix A (Table A.2). A second order non-linear regression equation was fitted between dependent and independent variables employing the experimental values. Following regression model was obtained to predict the yield (ml) of nutmeg mace essential oil.

Oil yield = 5.08 -0.35A +0.35B + 0.050C -0.18 AB -0.13AC -0.32 BC -0.0025

A^2 -0.60 B^2 + 0.30 C^2 …..(4.2)

From Table A.2, it is inferred that the values of R^2, R^2-adj and R^2-pred for the total yield of oil were 98.16, 95.78 and 92.30 per cent, respectively. The coefficient of determination (R^2) of the regression model for oil yield was 98.16 per cent which implies that the model could account 98.16 per cent variability in data. The R^2-pred (92.30 per cent) is in reasonable agreement with the R^2-adj (95.78 per cent). Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (23.349) value for total yield of oil indicates that the model can be used to predict the response within the design space. Therefore, second order model was adequate in describing the total yield of MAE essential oil.

It is evident from Equation (4.2) that the total yield of oil was in positive correlation with power density, soaking time and in negative correlation with solid: water ratio. Table A.2 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on total yield of oil at 1 per cent ($p<0.001$) level of significance as F- calculated value (41.38) was found greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.4.3 Energy Consumption

Energy consumption for extracting nutmeg mace essential oil obtained in various combinations of experiments are shown in Table 4.2. The energy consumption varied between 1.09 and 1.79 kWh. The least energy consumption was obtained for solid: water ratio of 1: 14, power density of 14.4 $W.g^{-1}$ and a soaking time of 4 h.

The ANOVA table for the response "Energy consumption" is shown in Appendix A (Table A.3). A second order non-linear regression equation was fitted between dependent and independent variables. The regression model obtained to predict the energy consumption for extracting nutmeg mace essential oil is as follows.

$$\text{Energy consumption} = 1.72 + 0.021A + 0.00375B - 0.21C + 0.010AB + 0.072AC - 0.052BC - 0.37A^2 - 0.24B^2 + 0.041C^2 \quad \text{.....(4.3)}$$

From Table A.3, it may be inferred that the values of R^2, R^2-adj and R^2-pred for the total energy consumption were 98.35, 96.22 and 96 per cent, respectively. The coefficient of determination (R^2) of the regression model for energy consumption was 98.35 per cent which implies that the model could account 98.35 per cent variability in data. The R^2-pred (96 per cent) is in reasonable agreement with the R^2-adj (96.22 per cent). Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (16.752) value for total energy consumption indicates that the model can be used to predict the response within the design space. Therefore, second order model was adequate in describing the total energy consumption of MAE essential oil.

It is evident from Equation (4.3) that the energy consumption was in positive correlation with solid: water ratio, power density and in negative correlation with soaking time. Table A.3 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on total energy consumption at 1 per cent ($p<0.001$) level of significance as F- calculated value (46.24) was found to be greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.5 DETERMINATION OF PHYSICAL QUALITY CHARACTERISTICS

The physical quality characteristics of the nutmeg mace essential oil extracted using conventional hydro distillation (HD) method and microwave assisted extraction (MAE) method are listed in Table 4.3.

Table 4.2. Physical quality characteristics of nutmeg mace essential oil

Sl. No.	Sample	Refractive index	Specific gravity	Solubility (v/v)	Colour		
					*L**	*a**	*b**
1.	$S_1 P_1 T_1$	1.480	0.8	1.4	1.29	-0.12	0.13
2.	$S_1 P_1 T_2$	1.482	0.4	0.8	3.62	-0.12	2.51
3.	$S_1 P_1 T_3$	1.482	0.9	0.7	0.75	-0.05	-0.15
4.	$S_1 P_2 T_1$	1.479	0.9	0.8	3.84	-0.09	2.74
5.	$S_1 P_2 T_2$	1.477	0.8	1.0	3.63	-0.03	3.39
6.	$S_1 P_2 T_3$	1.475	0.9	1.0	4.52	-0.29	3.83
7.	$S_1 P_3 T_1$	1.476	0.8	0.9	1.98	-0.08	-0.11
8.	$S_1 P_3 T_2$	1.464	1.1	0.8	3.61	-0.11	2.49
9.	$S_1 P_3 T_3$	1.470	0.9	1.0	3.47	-0.02	0.31
10.	$S_2 P_1 T_1$	1.486	0.7	0.9	4.12	-0.22	3.43
11.	$S_2 P_1 T_2$	1.483	0.9	0.6	2.93	0.01	-0.13
12.	$S_2 P_1 T_3$	1.482	0.6	1.0	4.48	-0.28	3.86
13.	$S_2 P_2 T_1$	1.475	1.0	1.6	3.12	-0.04	-0.07
14.	$S_2 P_2 T_2$	1.477	1.0	1.5	5.21	-0.42	3.98
15.	$S_2 P_2 T_3$	1.473	0.8	0.8	3.41	-0.19	0.47
16.	$S_2 P_3 T_1$	1.465	1.0	0.5	2.83	-0.05	1.96
17.	$S_2 P_3 T_2$	1.470	0.8	0.9	3.72	-0.03	0.34
18.	$S_2 P_3 T_3$	1.463	1.1	1.2	5.01	-0.35	3.71
19.	$S_3 P_1 T_1$	1.479	0.9	1.5	4.44	-0.05	0.43
20.	$S_3 P_1 T_2$	1.481	0.7	1.1	4.23	-0.26	3.65
21.	$S_3 P_1 T_3$	1.478	0.9	0.7	3.52	-0.09	-0.20
22.	$S_3 P_2 T_1$	1.477	1.0	0.7	3.21	-0.08	2.29
23.	$S_3 P_2 T_2$	1.477	0.9	1.1	4.16	0.00	0.28
24.	$S_3 P_2 T_3$	1.476	0.9	1.1	4.62	-0.33	3.89
25.	$S_3 P_3 T_1$	1.472	0.9	1.1	2.34	0.13	-0.10
26.	$S_3 P_3 T_2$	1.466	0.8	0.5	2.73	-0.05	1.86
27.	$S_3 P_3 T_3$	1.471	0.9	1.6	2.79	0.01	0.35
28.	HD	1.479	0.9	1.5	3.31	-0.04	-0.18

4.5.1 Refractive Index

The values of refractive index of nutmeg mace essential oil obtained in various experiments are shown in Table 4.3. The values of refractive index varied from 1.463 to 1.483. The values are close to the actual refractive index of nutmeg mace essential oil i.e. 1.4740 to 1.4880 obtained by conventional distillation method as reported by Burdock *et al.* (1995).

The ANOVA table for the response "refractive index" is given in Appendix B (Table B.1). The second order non-linear regression equation was used to relate between dependent and independent variables. Following model was fitted to predict the refractive index of nutmeg mace essential oil.

$$\text{Refractive index} = 1.48 - 0.009B - 0.0013C + 0.0007AB + 0.0007AC + 0.0005BC - 0.0006A^2 - 0.0033B^2 + 0.0001C^2 \quad \text{.....(4.4)}$$

From Table B.1, it is inferred that the values of R^2, R^2-adj and R^2-pred for the refractive index were 97.41, 94.09 and 83.07 per cent, respectively. The coefficient of determination (R^2) of the regression model for refractive index was 97.41 per cent which implies that the model could account for 97.41 per cent variability in data. Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (16.872) value for refractive index indicates that the model can be used to predict the response within the design space.

It is evident from Equation (4.4) that the refractive index of oil is not very much effected by the input variables. Table B.1 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on refractive index at 1 per cent ($p<0.001$) level of significance as F- calculated value (29.30) was found to be greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.5.2 Specific Gravity

The specific gravity of nutmeg mace essential oil obtained in various experiments were tabulated in Table 4.3. The values of specific gravity varied between 0.4 and 1.1. Similar results were also reported by Burdock *et al*. (1995).

The ANOVA table for the response "specific gravity" is given in Appendix B (Table B.2). The second order non-linear regression equation was used to relate between dependent and independent variables. Following model was used to predict the specific gravity of nutmeg mace essential oil.

$$\text{Specific gravity} = 0.92 + 0.013A + 0.2B - 0.012C - 0.15AB - 0.025AC + 0.050BC - 0.048A^2 - 0.12B^2 + 0.052C^2 \quad \text{.....(4.5)}$$

From Table B.2, it could be inferred that the values of R^2, R^2-adj and R^2-pred for the specific gravity were 97.98, 95.37 and 89.88 per cent, respectively. The coefficient of determination (R^2) of the regression model for refractive index was 97.98 per cent which implies that the model could account 97.98 per cent variability in data. Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (23.566) value for specific gravity indicates that the model can be used to predict the response within the design space.

It is evident from Equation (4.5) that the specific gravity of oil is not very much effected by the input variables. Table B.2 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on the specific gravity at 1 per cent ($p<0.001$) level of significance as F- calculated value (37.65) was found to be greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.5.3 Solubility

The solubility of nutmeg mace essential oil obtained in various experiments in 85 per cent ethanol were tabulated in Table 4.3. The values of solubility varied from

0.5 to 1.6 v/v. The solubility values obtained in MAE nutmeg mace essential oil were in close relation with HD nutmeg mace essential oil.

The ANOVA table for the response "solubility" is given in Appendix B (Table B.3). The second order non-linear regression equation was used to relate between dependent and independent variables. Following model was used to predict the solubility of nutmeg mace essential oil.

$$\text{Solubility} = 1.52 - 0.1B + 0.18C - 0.15AB + 0.05AC + 0.15BC - 0.36A^2 - 0.36B^2 - 0.26C^2 \quad \text{.....(4.6)}$$

From Table B.3, it is inferred that the values of R^2, R^2-adj and R^2-pred for the solubility were 97.48, 94.23 and 78.88 per cent, respectively. The coefficient of determination (R^2) of the regression model for solubility was 97.48 per cent which implies that the model could account 97.48 per cent variability in data. Lack of fit was insignificant and F-value suggested that the model was significant at 1 per cent and 5 per cent level of significance. The adequate precision (15.659) value for solubility indicates that the model can be used to predict the response within the design space.

It is evident from Equation (4.6) that the solubility of oil is not very much effected by the input variables. Table B.3 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on the solubility at 1 per cent ($p<0.001$) level of significance as F- calculated value (30.06) was found to be greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.5.4 Colour

The colour of nutmeg mace essential oil is determined by using Hunter lab colour flex meter as explained in section 3.6.4. The values of *L*, *a* and *b* obtained for various samples are given in Table 4.3.

The ANOVA tables for the responses L, a and b are shown in appendices Appendix B (Table B.4, B.5, B.6). The second order non-linear regression equation

were used to relate between dependent and independent variables. Following models were fitted to predict the colour of nutmeg mace essential oil.

$$L^* = 5.08 - 0.1A - 0.28B + 0.58C - 0.37AB + 0.18AC + 0.45BC - 0.8A^2 - 0.73B^2 - 0.24C^2 \quad \text{.....(4.7)}$$

$$a^* = -0.4 - 0.014A + 0.04B - 0.1C + 0.05AB - 0.013AC - 0.06BC + 0.15A^2 + 0.12B^2 + 0.055C^2 \quad \text{..... (4.8)}$$

$$b^* = 3.81 + 0.015A - 0.43B + 0.61C - 0.44AB + 0.13AC + 0.33BC - 0.62A^2 - 0.57B^2 - 0.005C^2 \quad \text{.....(4.9)}$$

From Table B.4, it could be inferred that the values of R^2, R^2-adj and R^2-pred for the '*L* (whiteness / darkness)' were 97.54, 94.38 and 82.30 per cent, respectively. The coefficient of determination (R^2) of the regression model for *L* was 97.54 per cent. From Table B.5, it could be inferred that the values of R^2, R^2-adj and R^2-pred for the '*a* (redness / greenness)' were 98.02, 95.47 and 82.03 per cent, respectively. The coefficient of determination (R^2) of the regression model for '*a*' was 98.02 per cent. Also, from Table B.6, it was inferred that the values of R^2, R^2-adj and R^2-pred for the '*b* (yellowness / blueness)' were 97.64, 94.60 and 74.30 per cent, respectively. The coefficient of determination (R^2) of the regression model for '*b*' was 97.64 per cent. For all the colour values, lack of fit was found to be insignificant and F-value suggested that the models were significant at 1 per cent and 5 per cent level of significance. Therefore, second order models were adequate in describing the *L*, *a* and *b* values of MAE essential oil.

It is evident from Equations (4.7), (4.8) and (4.9) that the colour of oil is not very much effected by the input variables. Tables B.4, B.5 and B.6 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on the L at 1 per cent ($p<0.001$) level of significance as F- calculated

value ((30.84), (38.50) and (32.17)) was found greater than the F table value (F_{tab} (9,7) = 6.71 (1 per cent) and 3.68 (5 per cent)).

4.6 OPTIMIZATION OF PROCESS PARAMETERS

4.6.1 Effect of Process Parameters on Extraction Time of Oil

The relationship between independent (Solid: water ratio, power density and soaking time) and dependent variables are illustrated by plotting 3D graphs representing the response (extraction time) surface generated by the model (Equation. 4.1). The 3D responses were shown in Fig. 4.1, comprising of three graphs a, b and c.

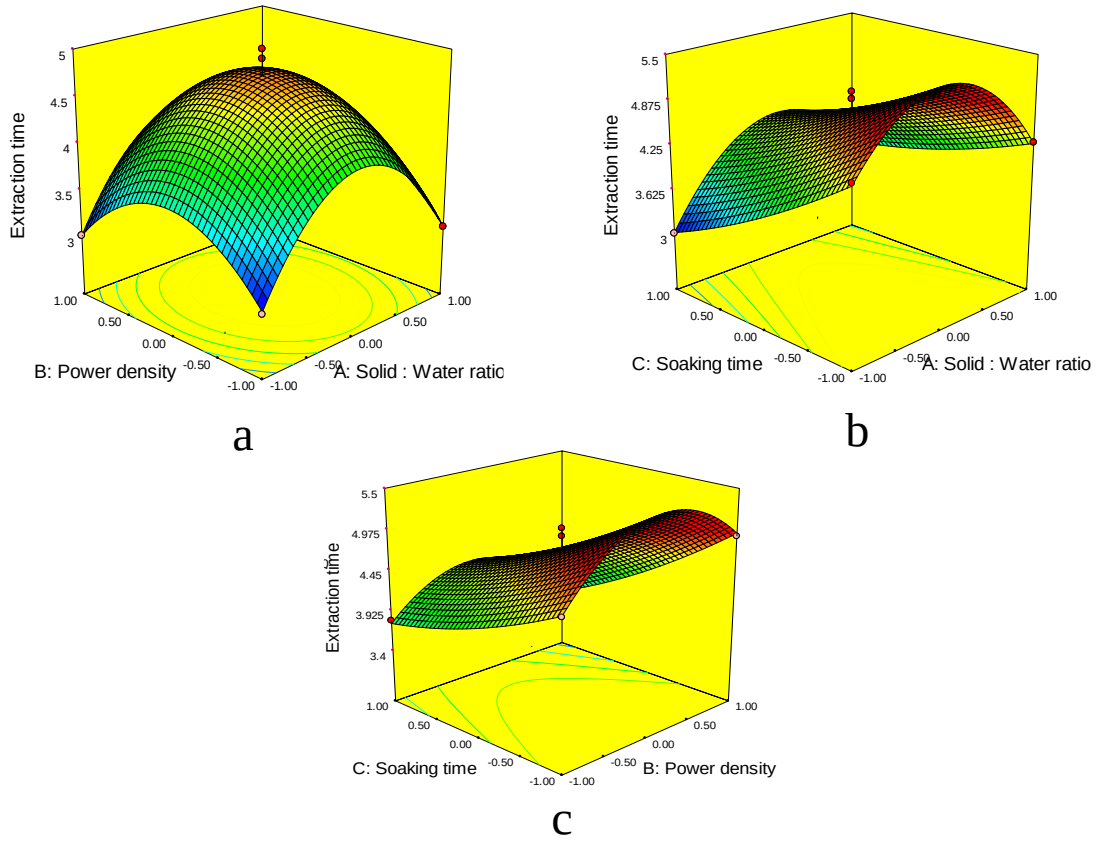


Figure 4.1. Effect of process parameters on extraction time

It is perceived from the Fig. 4.1 (a) and (b) that as solid: water ratio increases the total extraction time decreases. The total time of extraction varied from 3 h to 4 h 36 min. The least time consumption i.e. 3 h was obtained when the experiments were

performed with a solid: water ratio of 1: 14. It may be observed from the Fig. 4.1 (b) and (c) that as soaking time increases from 2 h to 4 h, there is only a slight decrement in the total extraction time. This indicates that soaking time has an insignificant effect on the total time of extraction. This was also supported by the surface plot in Fig. 4.1 (b) and (c) showing soaking time effect by a straight line. Similar findings were also reported by Gopika and Ghuman (2014) for celery seed essential oil.

Also, from Fig. 4.1 (a) and (c) it is concluded that at low power densities the extraction time was found to be high. As power density increases the total extraction time increases to a certain point and then gets decreased. The decrement in extraction time is due to the fact that, with increase in power level, the vapour pressure of water present inside the nutmeg mace increased leading to leaching out and evaporation of volatile oil along with vapour (Chemat *et al.*, 2006).

When compared with conventional hydro distillation process, the total time taken for extracting nutmeg mace essential oil from 50 g of sample was found to be 3 h. Whereas for the same oil yield hydro distillation process took 8 h. Therefore, it could be inferred that microwave assisted extraction was superior in terms of saving extraction time.

4.6.2 Effect of Process Parameters on Oil Yield

The relationship between Solid: water ratio, power density and soaking time on total yield of oil is illustrated by plotting 3D graphs representing the response surface generated by the model (Equation. 4.2). The 3D responses were shown in Fig. 4.2, (a), (b) and (c).

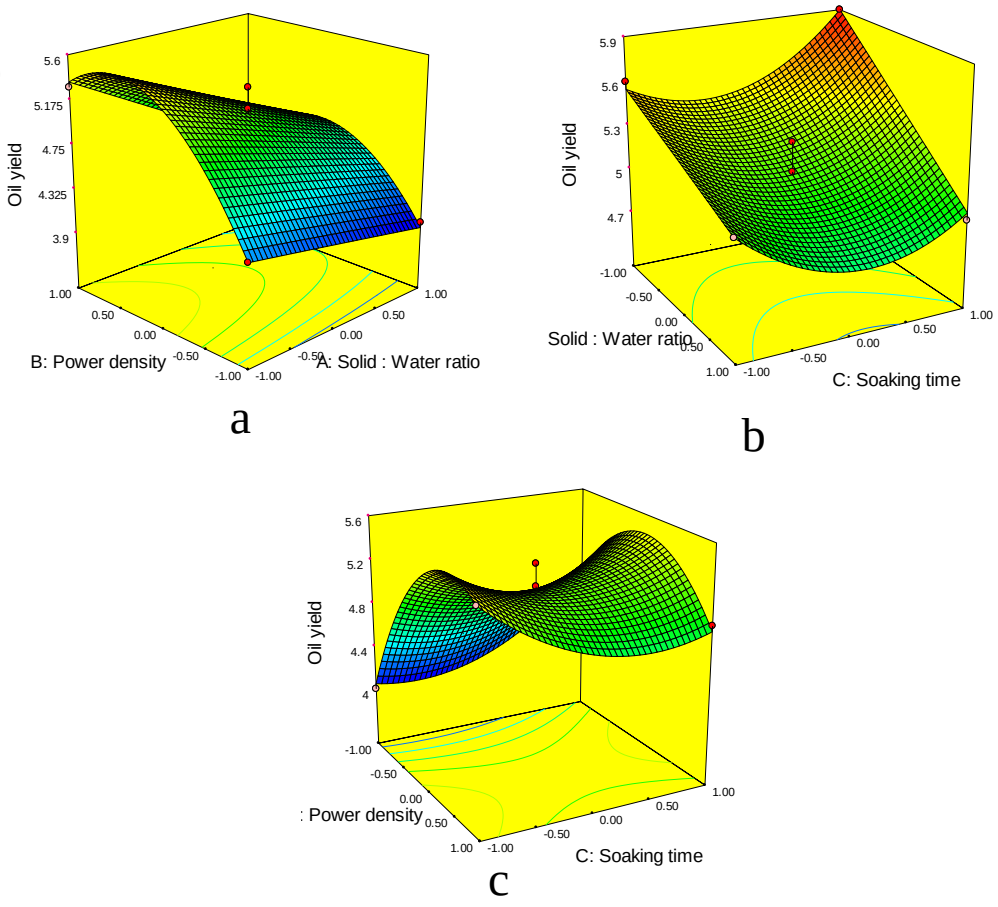


Figure 4.2. Effect of process parameters on oil yield

From Fig. 4.2 (a) and (c) it is concluded that power density has a significant effect on total yield of oil. At a low power density of 9.6 $W.g^{-1}$ the essential oil yield was found to be less. This trend might be due to the temperature being not enough to burst open the oil glands. With increase in power density, total yield of oil increased to a maximum of 5.9 ml at a power density of 14.4 $W.g^{-1}$. Further increase in power density leads to a decrease in the yield of essential oil. This decrease in yield of oil at higher power density is due to higher evaporation rate of essential oil than the condensation rate (Desai and Parikh, 2012).

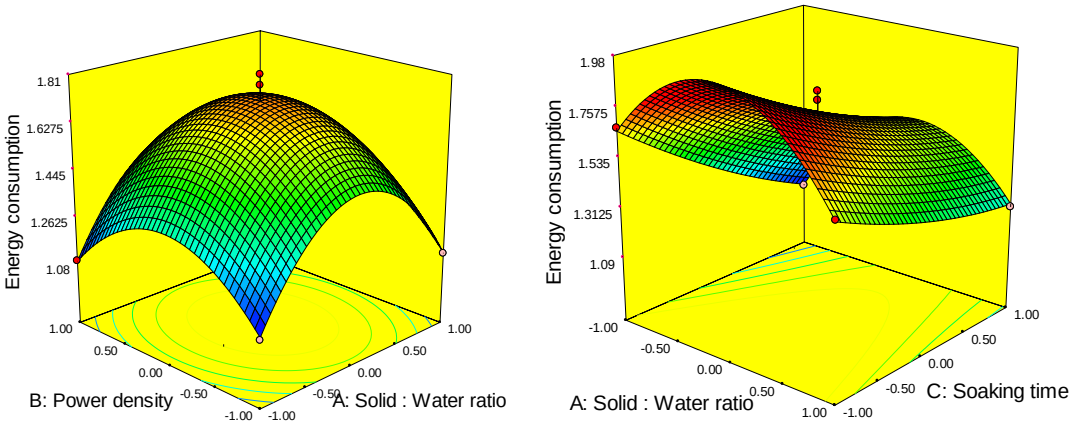
Fig. 4.2 (b) and (c) shows that soaking time has a significant effect on the yield of essential oil. Increase in soaking time leads to an increase in the yield of essential

oil up to a period of 3 h. Beyond 3 h there was not much effect in the yield of oil. Increase in soaking time leads to an increase in pressure inside the mace resulting in bursting of outer layers of mace. This bursting leads to release of oil, which increased the oil yield when compared to conventional hydro distillation process (Lucchesi *et al.*, 2007; Gopika and Ghuman, 2014).

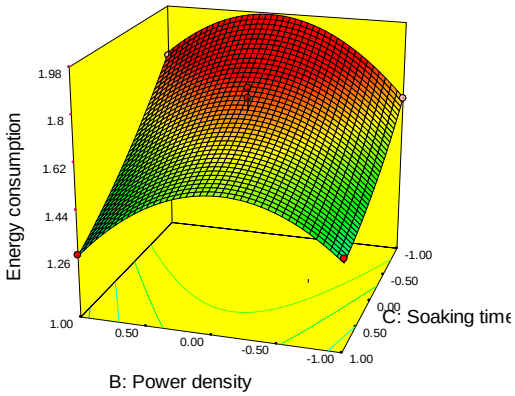
From Equation 4.2, it may be inferred that solid: water ratio had an insignificant effect on total yield of essential oil. This was also supported by surface plot in Fig. 4.2 (a) and (b) showing solid: water ratio effect by a straight line. When compared with conventional hydro distillation, the total yield of oil obtained in both the process were almost similar (Desai and Parikh, 2012; Chen *et al.*, 2016). About 10-12 per cent essential oil found in nutmeg mace through both process.

4.6.3 Effect of Process Parameters on Energy Consumption

The relationships of Solid: water ratio, power density and soaking time with that of the response 'energy consumption' are illustrated by plotting 3D surface graphs generated by the model (Equation. 4.3). The 3D responses were shown in Fig. 4.3, (a), (b) and (c).



a b



C

Figure 4.3. Effect of process parameters on energy consumption

Fig. 4.3 (a) and (c) shows that increase in power density increases total energy consumption up to a certain extent and then gets decreased. This might be due to increase in power density resulting in a decrease in total extraction time and thus to a decrease in total energy consumption (Desai and Parikh, 2012). When compared with hydro distillation the total energy consumed for microwave assisted extraction is 1.09 kWh whereas for the same oil yield HD process resulted in an energy consumption of 2.43 kWh.

It can be concluded from the Equation 4.3 that soaking time had insignificant effect on total energy consumption. This was also supported by surface plot in Fig. 4.3 (b) and (c) showing soaking time effect by a straight line. Solid: water ratio had a significant effect on the total energy consumption. Increase in solid: water ratio showed a decrease in total energy consumption, due to decrease in total extraction time.

4.6.4 Effect of Process Parameters on Refractive Index of Oil

The relationship between Solid: water ratio, power density and soaking time on refractive index are illustrated by plotting 3D graphs representing the response surface generated by the model (Equation. 4.4). The 3D responses were shown in Fig. 4.4, (a), (b) and (c).

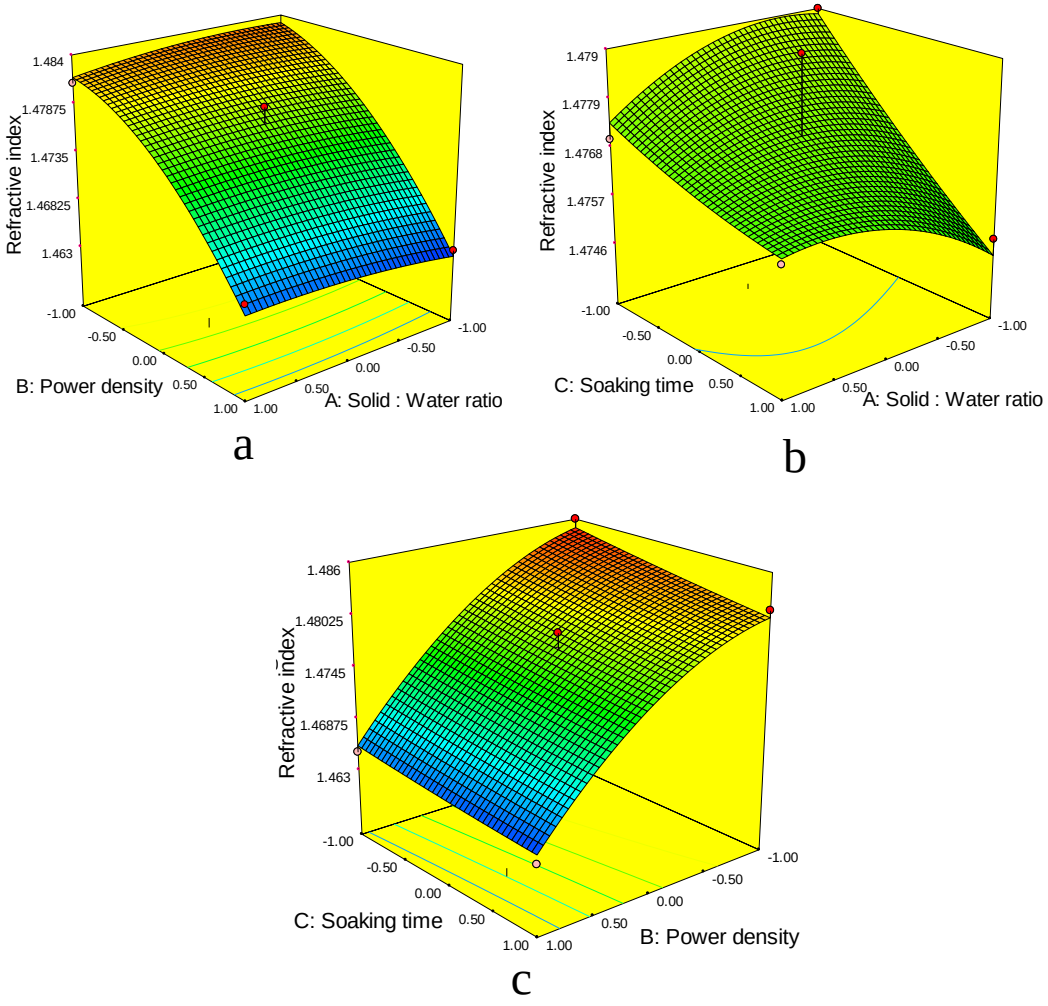


Figure 4.4. Effect of process parameters on refractive index of oil

The solid: water ratio was found to have no effect on the refractive index of the essential oil. The power density and soaking time have an insignificant effect on the refractive index of the oil. As the power density increases, the refractive index of oil has got decreased slightly. This might be due to the raise in temperature which increases speed of light in medium resulting in lower refractive index values, as refractive index is the ratio of speed of light in vacuum to the speed of light in medium (Anon., 2014). But when compared with the refractive index of hydro distilled oil, the refractive index of MAE essential oil also falls within the range of 1.474 to 1.4880.

Therefore, the refractive index was found to be similar for essential oil obtained in both the processes (Guan *et al.*, 2011).

4.6.5 Effect of Process Parameters on Specific Gravity of Oil

The relationship between Solid: water ratio, power density and soaking time on specific gravity are illustrated by plotting 3D graphs representing the response surface generated by the model (Equation. 4.5). The 3D responses were shown in Fig. 4.5, (a), (b) and (c).

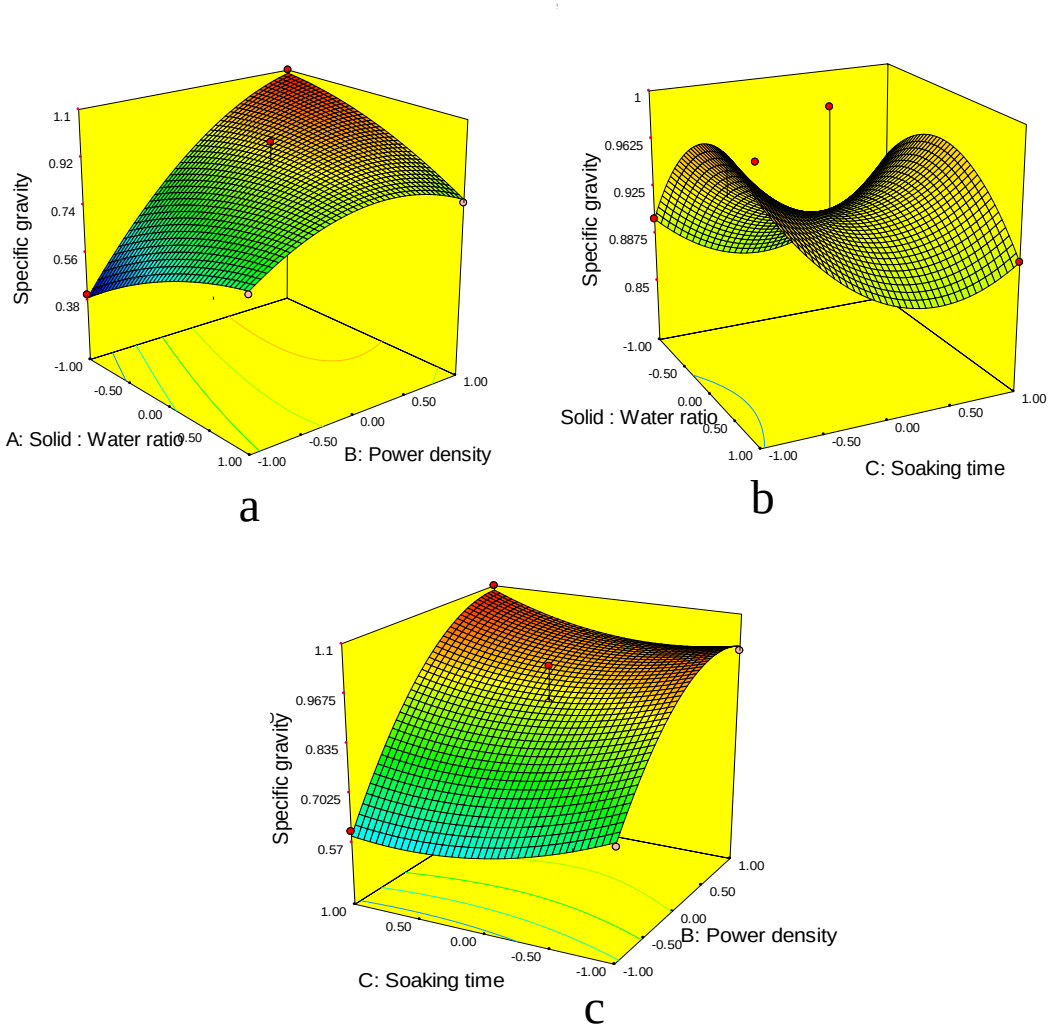


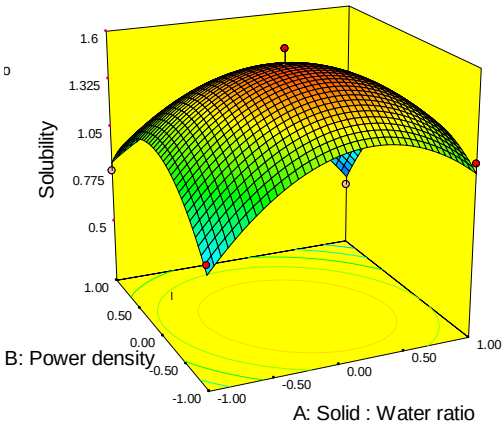
Figure 4.5. Effect of process parameters on specific gravity of oil

Equation 4.5 illustrates that solid: water ratio and power density had a significant effect on specific gravity of oil, whereas soaking time has an insignificant effect. From Fig. 4.5 (a) and (c) it may be revealed that specific gravity increases with increase in power density. Burdock *et al.* (1995) had reported specific gravity values of hydro distilled nutmeg mace essential oil. No significant difference in specific gravity values were observed between the MAE mace oil and conventional hydro distilled oil as found experimentally and the reported values.

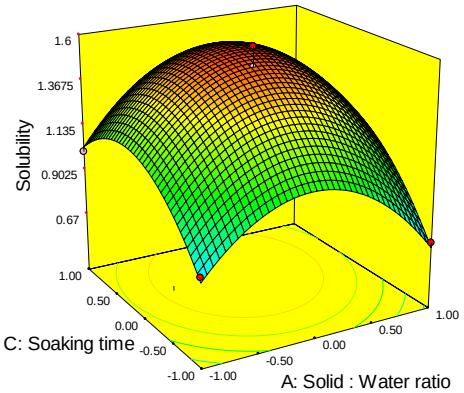
4.6.6 Effect of Process Parameters on Solubility of Oil

The variation of Solid: water ratio, power density and soaking time with that of solubility is shown by plotting 3D graphs representing the response surface generated by the model (Equation. 4.6). The 3D responses were shown in Fig. 4.6, (a), (b) and (c).

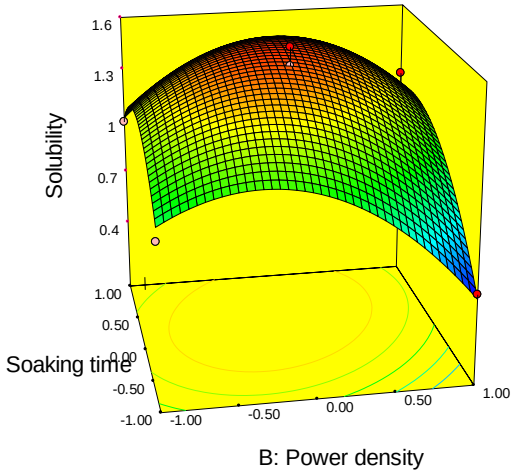
From Equation 4.6 it may be perceived that solid: water ratio has no effect on solubility of essential oil, whereas power density has negative effect and soaking time has positive effect on the solubility of nutmeg mace essential oil. However, there is no much difference between the solubility values of oil obtained by HD and MAE processes. Similar results were also reported by Golmakani *et al.* (2008) for thyme essential oil.



a



b

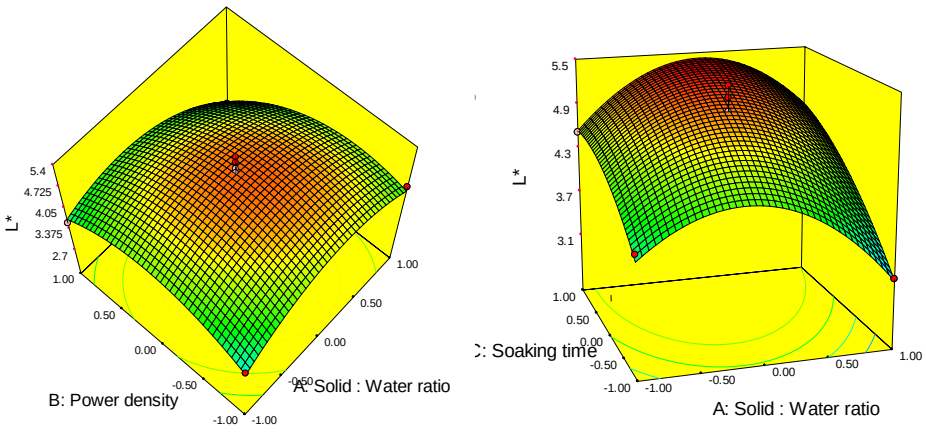


c

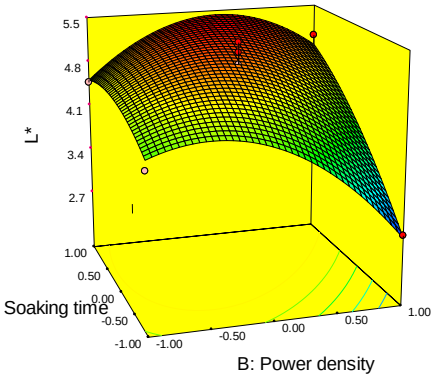
Figure 4.6. Effect of process parameters on solubility of oil

4.6.7 Effect of Process Parameters on Colour of Oil

The effect of Solid: water ratio, power density and soaking time on the colour of the nutmeg mace essential oil is illustrated by plotting 3D graphs representing the response surface generated by the model Equations 4.7, 4.8 and 4.9. The 3D responses for *L**, *a** and *b** were shown in Fig. 4.7, 4.8 and 4.9 comprising of three graphs a, b and c each.



a b



c

Figure 4.7. Effect of process parameters on lightness of oil

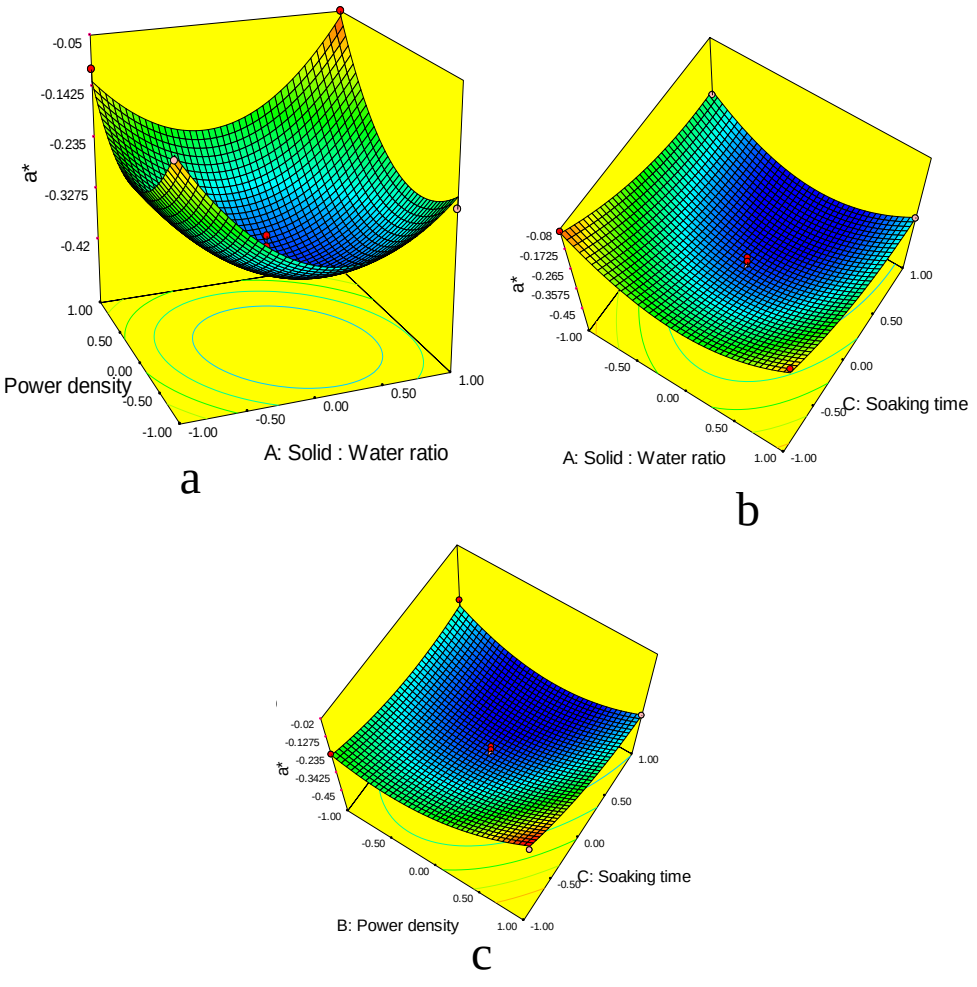


Figure 4.8. Effect of process parameters on *a**

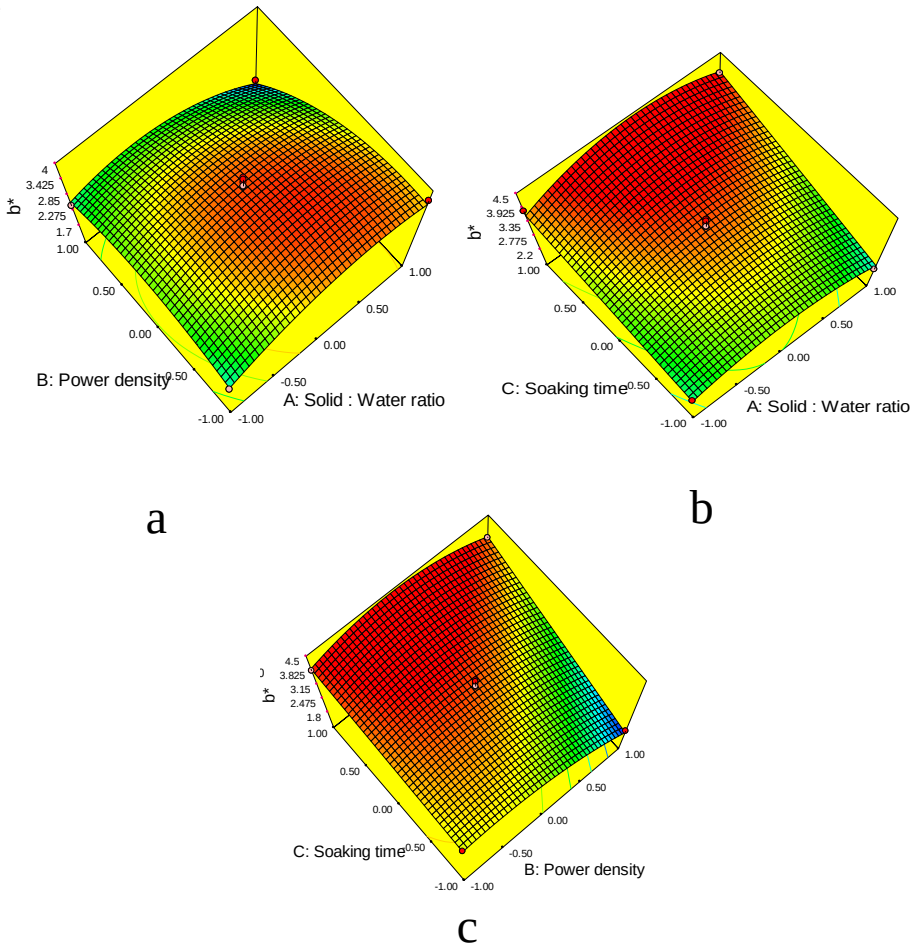
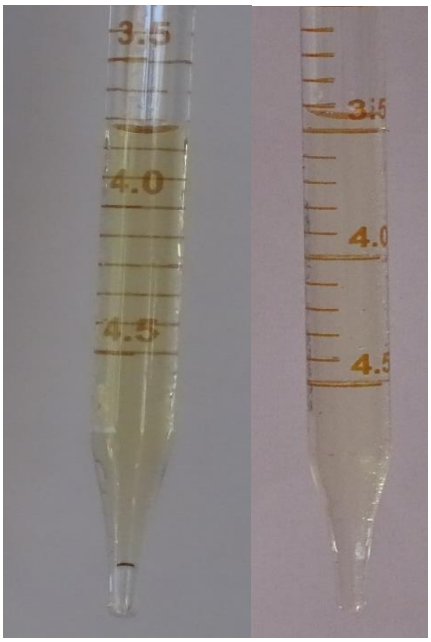


Figure 4.9. Effect of process parameters on *b**

From Fig. 4.9 (a), (b) and (c), it is concluded that the process parameters had a significant effect on the '*b*' value of essential oil. Increase in power density leads to a slight increase in '*b*' values which indicates that the oil has attained yellow colour. This might be due to the reason that with increase in power density, the colour of oil changes to dark yellow or even black due to the presence of suspended material (Baron and Villa, 2014).

Further it may be inferred from the surface plots 4.7 and 4.8 that the process parameters have insignificant effect on the '*L*' and '*a*' values. This indicates that clear oil is obtained by microwave assisted process. The essential oil obtained by hydro

distillation process is a clear liquid, whereas in microwave assisted process it is pale yellow clear liquid. Plate 4.1 shows the difference between the oils obtained by both the processes.



MAE HD

Plate 4.1. Nutmeg mace essential oil extracted through hydro distillation & microwave assisted process

4.6.8 Desirability

Desirability analysis was performed by employing the design expert software. Desirability ranges from zero to one for any given response. The program combines individual desirabilities into a single number and then searches for the greatest overall desirability. A value of one represents the ideal case. A zero indicates that one or more responses fall outside desirable limits (Myers *et al.*, 2009).

From the desirability analysis, the optimal level of various parameters were found and listed in Table 4.4. From the analysis a Solid: water ratio of 1: 14; Power

density of 14.4 $W.g^{-1}$; and Soaking time of 4 h were found to be the optimum values. The extraction time, yield of nutmeg mace oil and energy consumption at this optimum process parameter levels for microwave assisted process were found to be 3 h, 5.9 ml/50 g sample, and 1.09 kWh, respectively whereas the same were found to be 8 h, 5.8 ml/ 50 g sample and 2.43 kWh, respectively for conventional hydro distillation process. These results clearly indicate that for same oil yield, the microwave assisted process resulted in a very rapid extraction process with considerable saving in energy. The saving in time and energy of the process was found to be 62.50 and 55.14 per cent, respectively. The desirability of the optimization was found to be 0.887. As the desirability value is close to 1.0, the optimized values could be considered ideal.

Table 4.3. Optimal level obtained from the desirability analysis

Sl. No.	Response	Desirability	Optimal level	Low level	High level
1.	Extraction time	Minimize	3	3	5
2.	Oil yield	Maximize	5.9	4	5.9
3.	Energy consumption	Minimize	1.09	1.09	1.81
4.	Refractive index	Is in range	1.475	1.474	1.488
5.	Specific gravity	Is in range	0.9	0.88	0.93
6.	Solubility	Is in range	1.0	0	3
7.	L*	Maximize	4.52	2.73	5.36
8.	a*	Minimize	-0.29	-0.42	0.5
9.	b*	Maximize	3.83	1.86	3.98

4.7 CHEMICAL ANALYSIS

The main active component present in nutmeg mace essential oil is myristicin which is usually taken as a standard for comparison. The presence of myristicin in MAE oil under optimized process values was determined by gas chromatography method. The presence of myristicin was analyzed by comparing with the chromatograph of myristicin standard (Fig. 4.10). The chromatograph of the optimally produced MAE nutmeg mace essential oil is shown in Fig. 4.11. The myristicin content was then compared with chromatograph of hydro distilled nutmeg mace oil. The chromatograph of HD nutmeg mace oil is shown in Fig. 4.12.

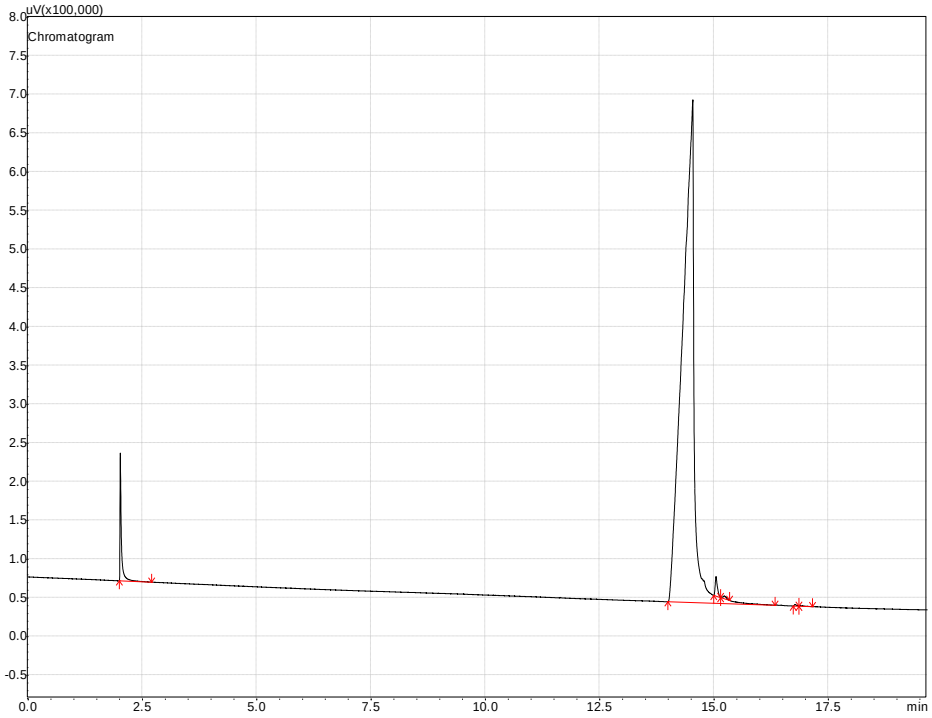


Figure 4.10. Gas chromatograph of myristicin standard

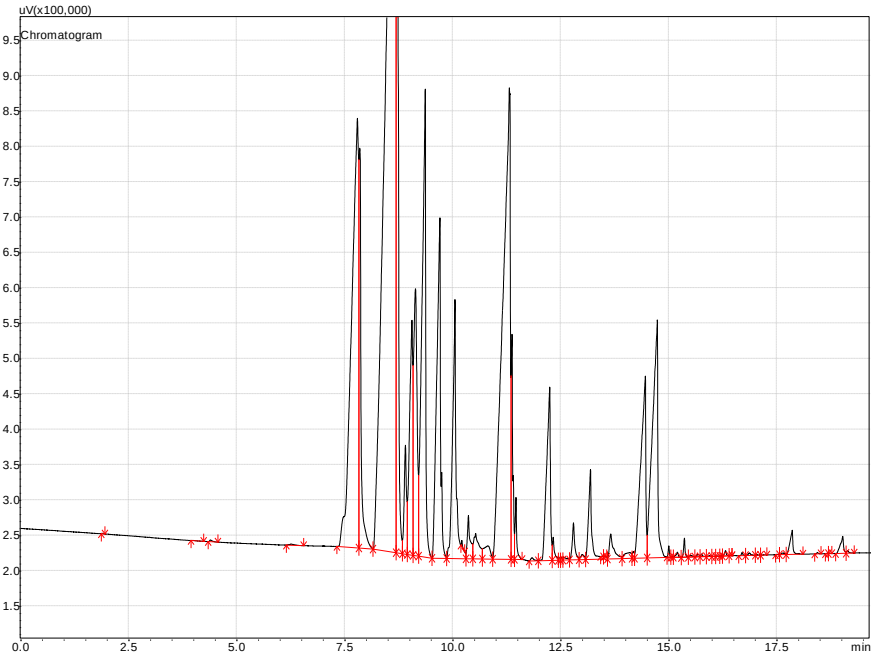


Figure 4.11. Gas chromatograph of MAE nutmeg mace oil

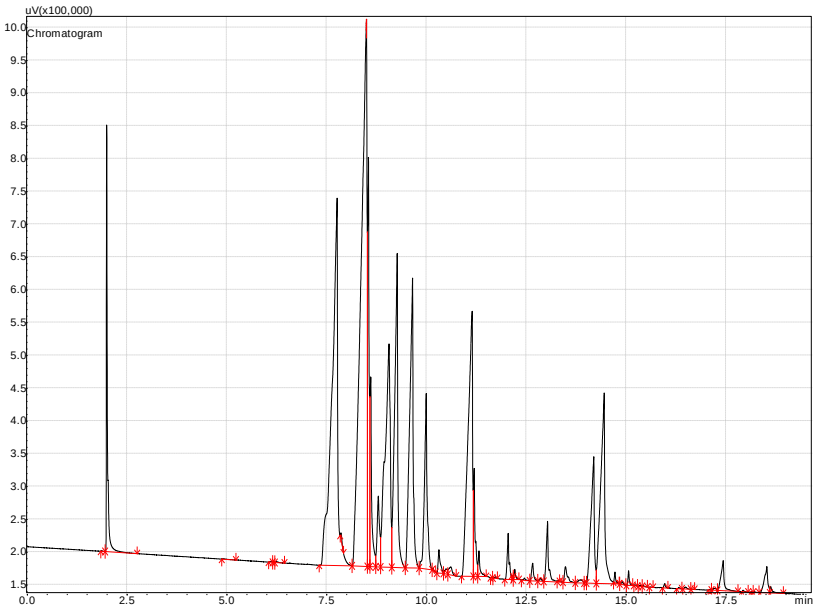


Figure 4.12. Gas chromatograph of HD nutmeg mace oil

From the Fig. 4.11 and 4.12, it may be concluded that the peak of myristicin in the chromatograph of MAE nutmeg oil at optimized conditions is slightly higher compared to the peak of myristicin in the chromatograph of HD nutmeg oil. This might be due to the degradation of myristicin at high temperatures of around 100^{o}C in HD process. Also, the myristicin content of MAE sample was slightly higher due to low power density and lesser extraction time because of which the oil was not exposed to high temperatures for a long time resulting in lower degradation of thermally liable myristicin.

CHAPTER V

SUMMARY AND CONCLUSION

Spices are mainly used for flavour, colour, aroma and preservation of food or beverages. They are obtained from plant or vegetable products or mixtures of both. Essential oils which are the volatile components distilled from the aromatic plant materials, gives spices their specific scent and flavour. The essential oils have gained their importance in therapeutic, cosmetic, aromatic, fragrant and spiritual uses. They are generally extracted by distillation.

The commonly used distillation methods are hydro distillation, steam distillation, solvent extraction, cold pressing etc. But these methods carry the disadvantages mainly deals with the quality of final product such as loss of some volatile notes, low extraction efficiency and degradation of unsaturated ester compounds through thermal or hydrolytic effects. These processes also requires high extraction times and energy consumption.

A recent modification of the essential oil extraction is the microwave assisted process. In microwave heating of food materials, the internal heating of the already present water within the plant material by the microwaves leads to the rupture of the glands and odoriferous receptacles freeing the essential oil which is then evaporated by the in-situ water of the plant material. The water then evaporated could then be passed through a condenser outside the microwave cavity where it is condensed. It has been found that the use of microwaves for extraction of active components could result in enhanced performance in terms of quality and quantity such as high extraction and efficiency, less extraction time and increased yield with quality of the extracted oil superior to that of other conventional methods due to the mild conditions. Since the plant material respond differently to the action of microwaves, the process parameters needs to be standardized for each biomaterial subjected to the kinetic effect of microwave action.

This study envisages development of a microwave assisted extraction system for extracting nutmeg mace essential oil. The developed extraction system consists of a microwave cavity, extraction unit, supporting stand and energy meter. A microwave oven with maximum microwave power delivery of 1200 W was chosen as microwave source. The oven consists of a control panel where cooking time, power, action indicators and clock time are displayed and controlled. The oil is extracted by micro mode since the temperatures are low at this mode. The Extraction unit consists of a Clevenger hydro distillation system in which recycling of distilled water also takes place. A hole of ½ inch diameter was drilled on the top of the microwave cavity, and a glass tube of same diameter with both ends open is inserted into the hole of microwave cavity which acts as carrier of vapors from round bottomed flask which is inside the cavity to the condenser outside the microwave cavity. A supporting stand is fabricated and is placed outside the oven for supporting the glass extraction unit. The stand comprises of a circular ring (outer diameter 48 mm, inner diameter 43mm and height 69 mm) and three stainless steel pipes (each of diameter 12.7 mm and length 80 mm) which acts as supporting legs for the ring. For keeping the distillation unit straight without tilting a rubber cork was inserted into the circular ring through which the glass stem passes. A single phase induction type energy meter was connected to the microwave assisted extraction system to measure the energy consumed during the distillation process.

In order to evaluate the developed system towards extraction of nutmeg mace essential oil, the process parameters like solid: water ratio, power density and soaking time which would influence the essential oil yield, extraction time and energy consumption were chosen as independent variables. The physical quality characteristics like refractive index, specific gravity, solubility and colour of essential oil were selected as dependent variables. Based on the preliminary studies the levels of process parameters were fixed as solid: water ratios of 1: 14, 1: 10 and 1: 6, power densities of 9.6, 14.4 and 19.2 $W.g^{-1}$ and soaking times of 2, 3 and 4 h.

The experiments were performed by taking 50 g of soaked nutmeg mace for a stipulated period of time as mentioned above into the round bottomed flask of the extraction unit. The microwave power level along with time is set in the control panel for various treatment conditions. Microwaves heat the water and vapors of water as well as essential oil in nutmeg mace gets vaporized and passes out of the microwave cavity through the distillation stem into the condenser. These vapors then passed through the condenser where they gets condensed and falls back into the bottom of the extraction unit where the oil and water gets separated and oil which is lighter than water settles on the top and water which is denser settles on the bottom. The oil is collected by means of a stopper provided on the extraction unit. The water is drained off and the essential oil thus collected is dehydrated with anhydrous sodium sulphate and stored at $2^{o}C$ in amber coloured glass bottles for further analysis.

Hydro distillation was performed as control for comparing the microwave assisted process. The physical quality characteristics of essential oil obtained by both the processes were measured and compared. For determining the myristicin content which is considered as a main chemical constituent in nutmeg mace oil, Gas chromatography was used.

For optimization of the process parameters and to check the sufficiency of the experimental design, the second order non-linear regression equation was fitted between dependent and independent variables. Analysis of variance (ANOVA) for the final predictive equation was carried out using Design Expert Software. RSM was adopted and Box-Behnken design of three variables and three levels, each with three centre point combinations was used. The response surface equation was optimized for the response variables using the above software.

The results showed that with increase in soaking time above 3 h, the total time of extraction was found to decrease. Also, soaking time beyond 3 h did not have much effect in the yield of oil. Increase in power density results in a decrease in total extraction time and thus to a decrease in total energy consumption. The process

parameters has an insignificant effect on the physical quality characteristics of the oil. Microwave assisted process resulted in an oil yield of 8-12 per cent, with an extraction time of 3 h and energy consumption of 1.09 kWh. The time taken for extracting the mace essential oil in hydro distillation process was around 8 h for the same oil yield, with an energy consumption of 2.43 kWh. This indicates that for the same oil yield, the microwave assisted process resulted in a rapid extraction process with about 55 per cent saving in energy when compared to hydro distillation process. The physical quality characteristics of oil in both the process was found to be similar whereas the chemical constituent i.e. myristicin content was slightly higher in microwave assisted process compared with hydro distillation method.

The optimized conditions of solid: water ratio, power density and soaking time for extracting nutmeg mace essential oil in microwave assisted process was found to be 1: 14, 14.4 $W.g^{-1}$ and 4 h, respectively. Therefore, microwave assisted extraction could be considered as an extraction technique that results in the production of high quality oil in higher quantity in less time with minimum energy consumption.

The following are suggestions for future research work on the microwave assisted extraction process.

1. Inserting temperature sensing device to measure temperature at various power levels
2. Use of high temperature resistive silicone rubber corks in place of normal rubber corks
3. Provision for measurement of heat losses which occur during the extraction process

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APPENDIX A

ANOVA of output characteristics of MAE system

Table A.1 ANOVA for extraction time

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	9.49	9	1.05	46.25	< 0.0001	significant
A-Solid : Water ratio	0.031	1	0.031	1.37	0.2799	
B-Power density	1.250E-003	1	1.250E-003	0.055	0.8215	
C-Soaking time	2.64	1	2.64	116.08	< 0.0001	
AB	2.500E-003	1	2.500E-003	0.11	0.7502	
AC	0.16	1	0.16	7.02	0.0329	
BC	0.090	1	0.090	3.95	0.0872	
A^2	4.36	1	4.36	191.31	< 0.0001	
B^2	1.88	1	1.88	82.33	< 0.0001	
C^2	0.049	1	0.049	2.14	0.1873	
Residual	0.16	7	0.023			
Lack of Fit	7.500E-003	3	2.500E-003	0.066	0.9752	not significant
Pure Error	0.15	4	0.038			
Cor Total	9.64	16				

R^2 =0.9835 Adj R^2 = 0.9622 Pred R^2 = 0.9629 Adeq precision = 16.735

Table A.2 ANOVA for oil yield

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	4.42	9	0.49	41.38	< 0.0001	significant
A-Solid : Water ratio	0.98	1	0.98	82.65	< 0.0001	
B-Power density	0.98	1	0.98	82.65	< 0.0001	
C-Soaking time	0.020	1	0.020	1.69	0.2352	
AB	0.12	1	0.12	10.33	0.0148	
AC	0.062	1	0.062	5.27	0.0553	
BC	0.42	1	0.42	35.63	0.0006	
A^2	2.632E-005	1	2.632E-005	2.219E-003	0.9637	
B^2	1.53	1	1.53	128.91	< 0.0001	
C^2	0.37	1	0.37	31.43	0.0008	
Residual	0.083	7	0.012			
Lack of Fit	0.015	3	5.000E-003	0.29	0.8288	not significant
Pure Error	0.068	4	0.017			
Cor Total	4.50	16				

R^2 =0.9816 Adj R^2 = 0.9578 Pred R^2 = 0.9230 Adeq precision = 23.349

Table A.3 ANOVA for energy consumption

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	1.23	9	0.14	46.24	< 0.0001	significant
A-Solid : Water ratio	3.613E-003	1	3.613E-003	1.22	0.3056	
B-Power density	1.125E-004	1	1.125E-004	0.038	0.8509	
C-Soaking time	0.34	1	0.34	116.45	< 0.0001	
AB	4.000E-004	1	4.000E-004	0.14	0.7239	
AC	0.021	1	0.021	7.11	0.0322	
BC	0.011	1	0.011	3.73	0.0948	
A^2	0.56	1	0.56	190.69	< 0.0001	
B^2	0.24	1	0.24	82.68	< 0.0001	
C^2	7.252E-003	1	7.252E-003	2.45	0.1614	
Residual	0.021	7	2.958E-003			
Lack of Fit	1.225E-003	3	4.083E-004	0.084	0.9653	not significant
Pure Error	0.019	4	4.870E-003			
Cor Total	1.25	16				

R^2 =0.9835 Adj R^2 = 0.9622 Pred R^2 = 0.9600 Adeq precision = 16.752

APPENDIX B

ANOVA of physical quality characteristics of MAE nutmeg mace oil

Table B.1 ANOVA for refractive index of oil

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	7.366E-004	9	8.184E-005	29.30	< 0.0001	significant
A-Solid : Water ratio	2.060E-018	1	2.060E-018	7.376E-013	1.0000	
B-Power density	6.661E-004	1	6.661E-004	238.51	< 0.0001	
C-Soaking time	1.513E-005	1	1.513E-005	5.42	0.0528	
AB	2.250E-006	1	2.250E-006	0.81	0.3992	
AC	2.250E-006	1	2.250E-006	0.81	0.3992	
BC	1.000E-006	1	1.000E-006	0.36	0.5684	
A^2	1.516E-006	1	1.516E-006	0.54	0.4853	
B^2	4.725E-005	1	4.725E-005	16.92	0.0045	
C^2	9.474E-008	1	9.474E-008	0.034	0.8591	
Residual	1.955E-005	7	2.793E-006			
Lack of Fit	6.750E-006	3	2.250E-006	0.70	0.5979	not significant
Pure Error	1.280E-005	4	3.200E-006			
Cor Total	7.561E-004	16				

R^2 =0.9741 Adj R^2 = 0.9409 Pred R^2 = 0.8307 Adeq precision = 16.872

Table B.2 ANOVA for specific gravity of oil

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	0.51	9	0.056	37.65	< 0.0001	significant
A-Solid : Water ratio	1.250E-003	1	1.250E-003	0.83	0.3917	
B-Power density	0.32	1	0.32	213.33	< 0.0001	
C-Soaking time	1.250E-003	1	1.250E-003	0.83	0.3917	
AB	0.090	1	0.090	60.00	0.0001	
AC	2.500E-003	1	2.500E-003	1.67	0.2377	
BC	0.010	1	0.010	6.67	0.0364	
A^2	9.500E-003	1	9.500E-003	6.33	0.0400	
B^2	0.063	1	0.063	42.12	0.0003	
C^2	0.012	1	0.012	7.74	0.0272	
Residual	0.011	7	1.500E-003			
Lack of Fit	2.500E-003	3	8.333E-004	0.42	0.7510	not significant
Pure Error	8.000E-003	4	2.000E-003			
Cor Total	0.52	16				

R^2 =0.9798 Adj R^2 = 0.9537 Pred R^2 = 0.8988 Adeq precision = 23.566

Table B.3 ANOVA for solubility of oil

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	2.05	9	0.23	30.06	< 0.0001	significant
A-Solid : Water ratio	0.000	1	0.000	0.000	1.0000	
B-Power density	0.080	1	0.080	10.57	0.0140	
C-Soaking time	0.25	1	0.25	32.36	0.0007	
AB	0.090	1	0.090	11.89	0.0107	
AC	0.010	1	0.010	1.32	0.2882	
BC	0.090	1	0.090	11.89	0.0107	
A^2	0.55	1	0.55	72.07	< 0.0001	
B^2	0.55	1	0.55	72.07	< 0.0001	
C^2	0.28	1	0.28	37.59	0.0005	
Residual	0.053	7	7.571E-003			
Lack of Fit	0.025	3	8.333E-003	1.19	0.4193	not significant
Pure Error	0.028	4	7.000E-003			
Cor Total	2.10	16				

R^2 =0.9748 Adj R^2 = 0.9423 Pred R^2 = 0.7888 Adeq precision = 15.659

Table B.4 ANOVA for L* of oil

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	10.58	9	1.18	30.84	< 0.0001	significant
A-Solid : Water ratio	0.080	1	0.080	2.10	0.1907	
B-Power density	0.64	1	0.64	16.89	0.0045	
C-Soaking time	2.68	1	2.68	70.28	< 0.0001	
AB	0.56	1	0.56	14.56	0.0066	
AC	0.13	1	0.13	3.49	0.1038	
BC	0.83	1	0.83	21.72	0.0023	
A^2	2.68	1	2.68	70.24	< 0.0001	
B^2	2.27	1	2.27	59.66	0.0001	
C^2	0.23	1	0.23	6.10	0.0429	
Residual	0.27	7	0.038			
Lack of Fit	0.10	3	0.035	0.85	0.5336	not significant
Pure Error	0.16	4	0.041			
Cor Total	10.85	16				

R^2 =0.9754 Adj R^2 = 0.9438 Pred R^2 = 0.8230 Adeq precision = 15.283

Table B.5 ANOVA for a* of oil

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	0.30	9	0.033	38.50	< 0.0001	significant
A-Solid : Water ratio	1.513E-003	1	1.513E-003	1.76	0.2259	
B-Power density	0.013	1	0.013	14.92	0.0062	
C-Soaking time	0.082	1	0.082	95.60	< 0.0001	
AB	0.010	1	0.010	11.66	0.0112	
AC	6.250E-004	1	6.250E-004	0.73	0.4216	
BC	0.014	1	0.014	16.79	0.0046	
A^2	0.089	1	0.089	103.55	< 0.0001	
B^2	0.058	1	0.058	68.05	< 0.0001	
C^2	0.013	1	0.013	14.98	0.0061	
Residual	6.005E-003	7	8.579E-004			
Lack of Fit	3.125E-003	3	1.042E-003	1.45	0.3545	not significant
Pure Error	2.880E-003	4	7.200E-004			
Cor Total	0.30	16				

R^2 =0.9802 Adj R^2 = 0.9547 Pred R^2 = 0.8203 Adeq precision = 16.660

Table B.6 ANOVA for b* of oil

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Model	8.85	9	0.98	32.17	< 0.0001	significant
A-Solid : Water ratio	1.800E-003	1	1.800E-003	0.059	0.8153	
B-Power density	1.47	1	1.47	48.09	0.0002	
C-Soaking time	2.96	1	2.96	96.94	< 0.0001	
AB	0.78	1	0.78	25.61	0.0015	
AC	0.065	1	0.065	2.13	0.1882	
BC	0.44	1	0.44	14.24	0.0069	
A^2	1.61	1	1.61	52.50	0.0002	
B^2	1.34	1	1.34	43.95	0.0003	
C^2	1.053E-004	1	1.053E-004	3.442E-003	0.9549	
Residual	0.21	7	0.031			
Lack of Fit	0.14	3	0.046	2.43	0.2052	not significant
Pure Error	0.076	4	0.019			
Cor Total	9.07	16				

R^2 =0.9764 Adj R^2 = 0.9460 Pred R^2 = 0.7430 Adeq precision = 16.226