MICROWAVE EXTRACTION OF ESSENTIAL OILS
(FROM BLACK PEPPER AND CORIANDER)
AT 2.46 GHz

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Fulfillment of the Requirements for the Degree of Master of Science in the
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Saskatoon, Saskatchewan S7N5A9 CANADA
by

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ABSTRACT

Essential oils are composed of a wide range of bioactive chemical compounds. They traditionally found application as flavour, fragrances and medicinal aroma. Today, the essential oils are sought-after for innumerable applications starting from markers for plant identifications to base for semi-synthesis of highly complex molecules. The extraction of highly delicate essential oils from plants remains a crucial step in all these applications. By using microwaves to mediate the extraction, it is possible to maintain mild conditions and effect superior extraction. However, apart from laboratory trials, essential oil extraction using microwave energy is largely an unexplored area. In the current work, an integrated procedure for microwave extraction followed by volatiles sampling and analysis from selected botanical raw materials (viz. black pepper, *Piper nigrum* and coriander *Coriandrum sativum*) was developed. There are two problems to overcome in the extraction from solid plant materials: that of releasing the essential oil from solid matrix and letting it diffuse out successfully in a manner that can be scaled-up to industrial volumes. Towards this end, an innovative volatiles extraction unit was conceived, designed and developed that used thin layer, for microwave exposure and rotational mixing, to mitigate the effects of thermal gradient and non-uniform exposure of bulk matter.

The effect of varying the microwave field on the essential oils extracted was studied. The microwave field that coupled in the region of extraction was estimated from temperature rise measurement using the microwave power equation (with water as reference dielectric). The essential oil extracted under different microwave fields were compared using gas chromatography-mass spectrometry (GC-MS) and data analysis with SAS statistical software.

The microwave field at the site of extraction was sensed by symmetrical placement of biomaterial sample and a reference, in a rotational extractor, such that they both couple the same field during tumbling motion. By measuring the temperature rise in the reference accurately, it is possible to estimate the microwave field present at that position. The rotational extractor has a second degree of freedom, in that it can slide along the axel taking discrete positions. Each position leads to a different microwave exposure of the sample. It is possible to measure the relative variation of microwave field using temperature rise data at each position. It was found that, at position labeled R4, located at 65.6 mm from the right extreme of the microwave cavity had the highest effective microwave field strength of value 92.7 V/m.

The volatiles released from the biomaterials, black pepper and coriander, were sampled using solid phase micro extraction and analyzed using gas chromatography-mass spectrometry. The highest peaks representing beta-caryophyllene in black pepper and linalool in coriander were identified using mass spectrometric peak matching using NIST library.The extract (in terms of ion count) for each microwave parameter (such as field, water content level and solvent type) was plotted as a trend graph.

The current experiment successfully tested the procedure for following the microwave process in the extraction of sensitive spice volatiles (from black pepper and coriander). With the microwave field measured at the region of extraction, it was possible to plot pepper extraction versus the microwave field to which the pepper sample was exposed. The extraction was represented in terms of cumulative value of ion counts obtained in GC-MS analysis. This unique procedure developed in the current research allows for the graphical comparison of the microwave extractions. It was found that black pepper has a better response to microwave extraction than coriander. The pepper extraction was found to increase proportionately with increase in microwave field strength. The extraction was also enhanced proportionately by the incremental addition of water content at constant microwave field.

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DEDICATION

I dedicate this thesis to my wife Subhashini and daughter Harini who encouraged me enormously

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SYMBOLS AND ABBREVIATIONS

C_p	specific heat of water/biomaterial $kJkg^{-1}K$
c	velocity of light $3\times10^{8}ms^{-1}$
∂	or D_p penetration depth m
E	electric field intensity Vm^{-1}
H	magnetic field
$j=\sqrt{-1}$	
m	mass Kg
P	power absorbed W
Q	heat J
T	temperature K
V	volume m^{3}
z	axis taken as direction of propagation of electromagnetic wave
t	time s
v	velocity of propagation ms^{-1}
ε	absolute electric permittivity of the medium Fm^{-1}
ε''	dielectric loss
μ	absolute magnetic susceptibility of the medium Hm^{-1}
μ_0	the absolute magnetic susceptibility of free space = 1.26 x 10^{-6} Hm^{-1}
ε_0	the absolute electric permittivity of free space = 8.86 x 10^{-6} Fm^{-1}
ε_r	is the relative permittivity taken as 1 for biomaterials
μ_r	is the relative susceptibility usually taken as 1 for biomaterials
k	phase constant m^{-1}
λ	wavelength m
υ	frequency cycles s^{-1}
η	refractive index

α	attenuation constant dBm^{-1}
β	phase constant in a conducting dielectric $\deg m^{-1}$
σ	conductivity
ρ	bulk density kgm^{-3}
ω	angular frequency $rad \cdot s^{-1}$

Abbreviations

ASE: accelerated extraction
GC-MS: Gas Chromatography-Mass Spectrometry
GPE: gum phase extraction
HSSE: Head Space Sorptive extraction
PDMS: polydimethylsiloxane
SPE: solid phase extraction
SPME: Solid Phase Micro Extraction

SI units and symbols

Name	Symbol	Dimensions	Quantity
ampere (SI base unit)	A	A	Current
coulomb	C	A·s	Electric charge, Quantity of electricity
volt	V	$J/C = kg\cdot m^{2}\cdot s^{-3}\cdot A^{-1}$	Potential difference
ohm	Ω	$V/A = kg\cdot m^{2}\cdot s-3\cdot A^{-2}$	Resistance, Impedance, Reactance
ohm metre	Ω·m	$kg\cdot m^{3}\cdot s^{-3}\cdot A^{-2}$	Resistivity
watt	W	$V\cdot A = kg\cdot m^{2}\cdot s^{-3}$	Electrical power
farad	F	$C/V = kg^{-1}\cdot m^{-2}\cdot A^{2}\cdot s^{4}$	Capacitance
farad per metre	F/m	$kg^{-1}\cdot m^{-3}\cdot A^{2}\cdot s^{4}$	Permittivity
reciprocal farad	F−1	$kg^{1}\cdot m^{2}\cdot A^{-2}\cdot s^{-4}$	Elastance
siemens	S	$\Omega^{-1} = kg^{-1}\cdot m^{-2}\cdot s^{3}\cdot A^{2}$	Conductance, Admittance, Susceptance
siemens per metre	S/m	$kg^{-1}\cdot m^{-3}\cdot s^{3}\cdot A^{2}$	Conductivity
weber	Wb	$V\cdot s = kg\cdot m^{2}\cdot s^{-2}\cdot A^{-1}$	Magnetic flux
tesla	T	$Wb/m^{2} = kg\cdot s^{-2}\cdot A^{-1}$	Magnetic flux density
ampere per metre	A/m	$m^{-1}\cdot A$	magnetic induction
ampere-turns per weber	A/Wb	$kg^{-1}\cdot m^{-2}\cdot s^{2}\cdot A^{2}$	Reluctance
henry	H	$Wb/A = V\cdot s/A = kg\cdot m^{2}\cdot s^{-2}\cdot A^{-2}$	Inductance
henry per metre	H/m	$kg\cdot m\cdot s^{-2}\cdot A^{-2}$	Permeability
(dimensionless)	χ	-	Magnetic susceptibility

1. INTRODUCTION

The design of environmentally friendly process for essential oil extraction conceived for current research was among the very few its kind in reported literature when the project began in August 2003 (Ramanadhan 2003)*. Since then, there has been a few publications along very close lines (Chemat 2004, Milestone 2005) as discussed in the literature survey. The field of essential oils is vast and there appears to be great potential for inter-disciplinary systems research here (Figure 1.1).

The current research was carried out to develop a method for sensing the microwave process, measuring the microwave field at the region of processing and comparing the influence of different process parameters on the extraction. The research involved the following phases of work:

- standardizing extraction, sampling and analysis of essential oils using microwaves;
- studying the effect of microwave field variation on dry pepper extraction;
- studying the effect of microwave field variation on dry coriander extraction;
- comparing coriander and pepper extractions; and
- studying the effect of sample treatment on black pepper extraction.

This chapter briefly introduces the concept of microwave processing leading to extraction and analysis of plant volatiles.

*Note: The technology transfer division of the University of Saskatchewan has come forward to evaluate the current design for patenting and scale-up (ILO 2005).

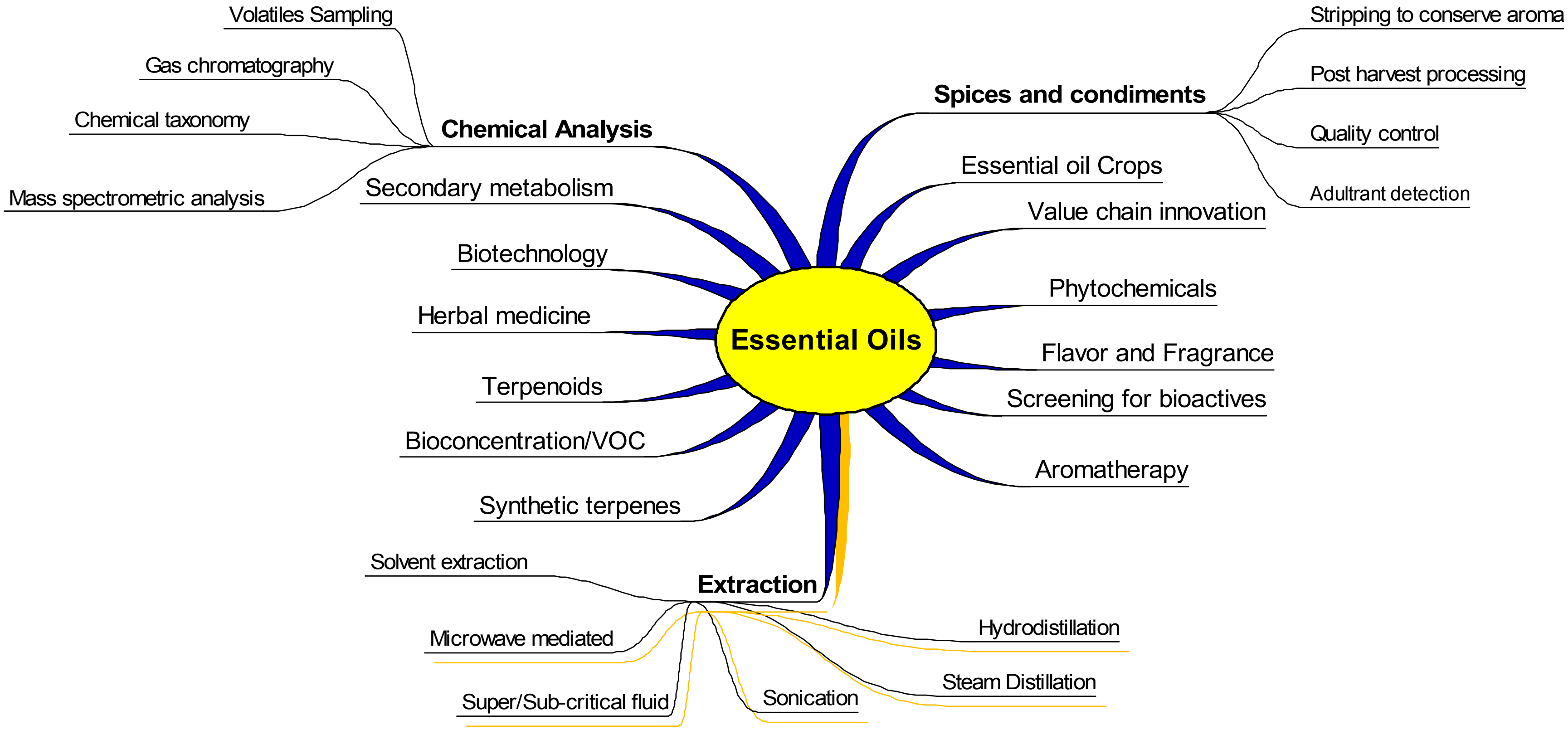


Figure 1.1 Tree diagram showing the wide branching of specializations in the field of essential oils.

The order of topics presented in this chapter is as follows:

Initially, the physical nature of microwave technology and its advantages are introduced. This is followed by the offline sensing method to monitor microwave extraction. This chapter introduces a systems approach made in current research to overcome several challenges arising out of the inherent nature of microwave processing and its relative infancy as an industrial technology.

Propagation of electromagnetic waves through lossy (absorbing) medium gives rise to heat. This heating phenomenon seen in cases of microwave coupling is called volumetric heating and can be ideal for sensitive application of heat to extract volatiles from biomaterials. Both the sensitive microwave extraction process as well as related measurements are emerging areas.

1.1. Microwave Technology - An Overview

Microwave energy is a superior alternative to several thermal applications owing to its efficient volumetric heat production. A comparative table of various heating applications is given in Table 1.1.

Table 1.1 Comparison of conversion efficiencies of various heating sources (Wilson 2003).

Appliance	**Temp** (°C)	**Appliance rating** (W)	**Time**	**Energy used** (kWh)	**Energy cost** US $
Electric oven	177	2000	1hr	2	0.17
Convection oven	163	1853	45 min	1.39	0.12
Gas oven	177	36	1 hr	3.57	0.07
Frying pan	216	900	1 hr	0.9	0.07
Toaster oven	218	1140	50 min	0.95	0.08
Crockpot	93	100	7 hr	0.7	0.06
Microwave oven	High	1440	15 min	0.36	0.03

The volumetric heating or heating of the bulk as opposed to transferring heat from the surface, inwards, is more efficient, uniform and less prone to overkill or supererogation. Controllability is by far the greatest advantage of microwaves over conventional thermal technologies. In processing applications, the ability to instantaneously shut the heat source makes enormous difference to the product quality and hence the production economics. The very nature of heating through the involvement of the raw material under processing (instead of using fossil fuels or less efficient, indirect electrical heating systems) brings about quality consistency as well as positive environmental impact. A detailed discussion of this is made in section 3.3 and 3.6.1.

Specifically in the essential oil extraction, microwave mediated processes are highly desirable due to their small equipment size (portability) and controllability through mild increments of heating. However, so far the microwave technology has found application in very few industrial bio-processing installations due to the lack of available data on microwave interaction with heterogeneous natural raw materials. The sensing and close control of microwave process is a challenging science. There is insufficient literature on microwave process sensing. This gap in experimental methodology for sensing microwave processes is addressed in the current research.

All the methods used in this research have been summarized in several comprehensive diagrams in chapter 5, which deals with materials and methods.

1.2 Microwaves are Electromagnetic Fields

Microwaves are electromagnetic fields in the frequency range 300 MHz to 300 GHz or between wavelengths of 1 cm and 1m (Singh 2001).

The electromagnetic field plays a central role in any attempt to describe physical reality. They are as real as the physical substances we ascribe to everyday experience. Therefore fields and particles of matter must be put on the same footing:

both carry energy and momentum, and both interact with the observable world (Rothwell 2001). Electromagnetic field is an oscillating electric and magnetic disturbance that spreads as a harmonic wave through space. The very low end of electromagnetic fields are felt at the charged double layers, direct current (DC) batteries and electrical conduction phenomena, whereas the higher end lead us to the realms of particle physics where matter transforms into radiation and vice versa.

Electromagnetic fields interact with matter resulting in energy transfer. Photosynthesis and human vision are examples of such interactions. There are numerous effects across the whole spectrum of electromagnetic frequencies most of which are not so obvious and appear only in numerical solutions.

The classical electromagnetism is described by Maxwell equations (Rothwell 2001). These describe essentially three electromagnetic properties viz., complex electrical permittivity and permeability (ability to store electrical and magnetic inductive capacity) and electrical conductivity.

Electromagnetic waves interact with matter in energy transfers that are quantized. Predominantly, waves in the microwave region excite molecular rotational energy levels (about 10^{-3} eV) and their energies fall just short of inducing Brownian motion in liquids and gases. They have an orienting effect on polar molecules present in microwave susceptible materials. This orienting effect, however, marginally falls short of synchrony with the alternating applied microwave field. This difference in synchrony, called dielectric relaxation results in a net absorption of energy, which manifests as heat.

1.3 Microwave Heating – Mathematical Aspects

The depth of the sample where the attenuation is 1/e (or in other words 1/α), can be approximately expressed as $(\lambda / 2\pi)(\sqrt{\varepsilon'} / \varepsilon'')$. This is the depth where effectively, the heat generated by an applied microwave field in a lossy medium is most likely to be volumetric.

In very basic sense, the heat generated by microwaves can be represented by the conduction equation as given in Eq. 1.1:

$$\rho c_p \frac{\partial T}{\partial t} = \lambda \nabla^2 T + Q \tag{1.1}$$

This simplistic case has to be expanded with terms for convection and transport in order to get an accurate numerical solution.

The source term for heat generation being in the form of *electromagnetic field* (microwave region), is a function of field frequency and absorbed power by foods.
The two equations used for deriving the field equations for microwave are the Ampere's law (Eq 1.2) and Faraday's Law (Eq 1.3) both of which are Maxwell's electromagnetic equations.

$$\nabla \times H = \sigma E + \frac{\partial D}{\partial t} \tag{1.2}$$

relates magnetic field **H** to the electric flux density **D**

$$\nabla \times E = \frac{\partial B}{\partial t} \tag{1.3}$$

relates electric field E to the flux density **B**.

However, a mode of the microwave can be chosen such that the magnetic component need not be considered. Equation 1.3 is used for non-magnetic materials. For a dielectric material, the propagation can be given as Eq 1.4

$$\nabla^2 E = \mu \varepsilon \frac{\partial^2 E}{\partial t^2} \tag{1.4}$$

Here μ is the permeability representing interaction with magnetic field and ε is the dielectric constant representing the interaction of non-conducting material with electric field. The power dissipated per unit volume is manifested as heat *Q*. Equation 1.5 is a concise statement for the heat generated in microwave (Meredith 1998):

$$Q = \frac{1}{2}\sigma_e \left|E\right|^2 \tag{1.5}$$

Q in the above equation appears as dissipation density in classical treatment of electromagnetism (where it is ohmic and applies equally to capacitive coupling as well as magnetic induction). The conductivity term σ includes the direct current conductivity (zero in this case) and an imaginary part of permittivity ε or the dielectric loss factor.

This is the starting point for any industrial microwave heating calculation, may it be for food or non-food application. However when foods are considered, there is a need to include innumerable influencing factors and corrections for unknowns. It is easier to model non-food processing. When it comes to foods, the basic difference is the fact that foods come in infinite variety, no two compositions and dimension remaining alike.

The rise in temperature is obtained by dividing the heat term Q by density and specific heat (Chang 2000). The functional density is difficult to measure for two reasons: (i) the density used in the permittivity measurements may not reflect the density of material exposed to multi-mode microwave radiation; and (ii) the density of the bulk may not be evenly and completely exposed to microwaves. Microwave penetrates to a limited depth, which is inversely proportional to the operating frequency as well as dielectric loss of the substrate.

Biomaterials heat up in the presence of microwaves owing to their chemical constituents that have dielectric relaxation. The heat generated by microwave interaction with biomaterials can be fully quantified by a measuring the frequency-dependent relative permittivity (in a vector network analyzer) or as an impedance spectrum, characterizing amplitude and time scale (via the relaxation time) of the charge-density fluctuations within the sample (in dielectric relaxation spectroscopy). The microwave processing literature uses relative permittivity measured using network analyzers as the indicator of the heat that can be generated in a sample when subjected to microwave.

In order to utilize microwave for unit processes, quantitative characterization has to be successfully carried out for: (a) electric field profile in the processing chamber; as well as, (b) the resulting changes occurring in the substrate. However, these are complex tasks, seldom achievable in industrial microwave equipments due to their poor reproducibility and thus giving rise to poor control over process quality.

Interpreting from the current literature on microwave processing, the complexity in controlling microwave process quality arises from the following:

1. The theoretical modeling of an empty multimode oven cavity has little predicting capability for events in a partially loaded cavity due to enormous perturbation of electric field by the very presence of a load.

2. The complexity of modeling is compounded by the fact that permittivity varies spatially depending on the chemical composition and bulk density of the load as well as temperature. Biomaterials can be by far the most complex substrates for microwave.

3. Low penetration depth of microwaves giving rise to competing heat transfer mechanisms. Uniform heating is rarely achievable in conventional microwave systems, often giving rise to both unprocessed and severely over heated spots.

4. Various mixing operations applied in mainstream thermal technologies become inapplicable in microwave systems due to material selection issues imposed by microwave environment.

The current research attempts to circumvent the above hurdles in a simple and scalable design towards better process quality control even in industrial scale. The microwave equipment used is a laboratory multimode microwave oven (COBER™, 1.2 kW capacity).

The two main challenges of this research are:

a) The design of an extractor that overcomes non-homogeneity in electric field profile of the microwave cavity and the non-uniform heating of materials caused by the shallow penetration of microwaves. This involves uniform and thin layer distribution of the substrate that can expose to a microwave field evenly. It is to be noted that both the spice particles as well as microwave field in an oven cavity are inherently non-uniform.

b) The design of experimental methods to follow the extraction after different periods of microwave exposure by characterizing the volatiles released and making a comparative study. This involves off-line sampling and chemical identification.

1.4 Microwave Extraction of Bioactives

Extraction is what brings us wake-up coffee. It is a trite but poorly understood engineering process. Solutes within the powdered raw material move or partition into the solvent phase and diffuse out of the solid matrix and eventually out of the particulate bulk. Traditionally, plant materials were subjected to mechanical shear to release the volatiles in virgin state. There are today various closely controlled sophisticated methods of extraction from distillation, through leaching to super- or sub-critical solvent extraction. Among the various available methods, microwave assisted extractions show the highest promise. Even among the microwave methods there are several variations. The literature survey covers some of the latest reported methods of microwave extraction.

Many proposed microwave based methods often adopt comminution for pre-treatment (Gaikar 2002). In industrial scale, Annatto (which gives cheddar cheese its golden hue), for instance, is extracted by particle attrition and impact using a ball mill (Mendonça 2001). The various methods used in conventional essential oil extraction are given in Figure. 3.8. Solvent extraction remains the most important methods of volatile extraction. From macerated species, there have been attempts to use various solvents to effect extraction. Among several new technologies, microwave-assisted solvent extraction is also well-reported (Nélida 1999; Gaikar 2002), but that is not where the green technological future is heading. Microwave ovens have gained acceptance as a mild and controllable processing tool. Microwaves allow simple, rapid and low solvent consuming processes. (Bernard 2002; Amer 1998). In the current research a study of extraction method from dry raw materials or with addition of water content without necessitating the use of organic solvents was studied.

1.5 Sampling and Analysis of Extracts

Sampling technology has advanced significantly. In our work we use the latest and highly accurate method, the solid phase micro extraction (SPME). The SPME is described in Figure 1.2.

There are indeed various options in the market. Some of the recent technologies include: gum phase extraction (GPE); solid phase extraction (SPE); head space sorptive extraction (HSSE); accelerated solvent extraction (ASE) etc. SPME however, is the most widely used method owing to its simplicity.

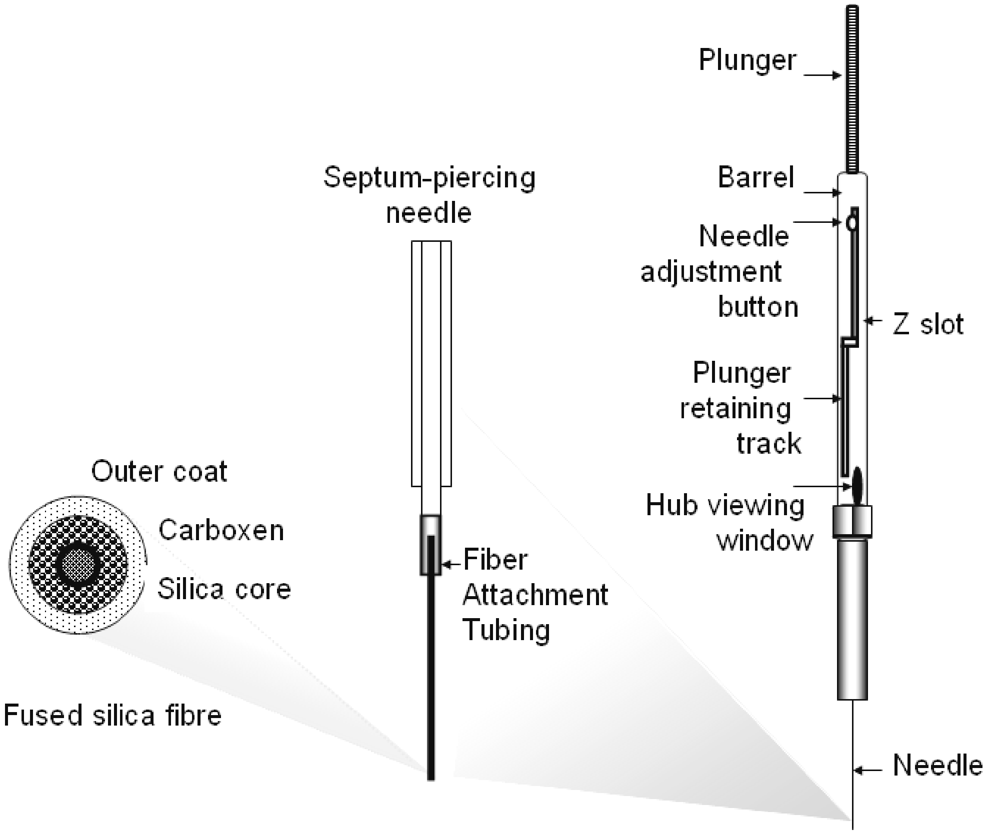


Figure. 1.2 The component details of solid phase micro extraction syringe.

Sorptive extraction involves extraction of organic compounds from a sample matrix into the bulk of a retaining phase. The mechanism is not like adsorption in charcoal or silica gel or tenax or polyurethane foam. It is based on partitioning or dissolution in an amorphous polymeric retaining phase that is above its glass transition temperature. In this method, the silicone rubber polydimethylsiloxane (PDMS) or PDMS/carboxen is used as the enrichment phase (sorbent). PDMS fiber looks amber in color; it has a feel like a nylon cable but a bit brittle at room temperature. PDMS is also the best known as GC stationary phase (Sandra 2002).

PDMS/gas distribution coefficients are known from GC retention times, PDMS/water distribution coefficients are very close to octanol/water and easy to calculate. PDMS is highly inert. It has sufficient thermal stability (from minus 100°C to 350°C). Its degradation products, if any, are non-interfering. When extractions are aimed at larger than sampling volumes, there are many limitations posed by the fragile nature of plant extracts. Most of the compounds expected to be detected in the current research fall below 150 atomic mass units (amu) and in this range, the carboxen fiber is reported to have the best sensitivity (Figure 1.3). This was the fiber that was used in the current research.

The procedure of SPME operation is straightforward. The steel needle of the SPME is pierced into the septum of the gas tight sample holder and a predetermined length of fiber is released for adsorption. After a set duration of adsorption, the SPME needle is released starting with the fiber followed by the needle. This sampling is stable and can last for a few hours. Once the gas chromatograph (GC) is ready for the run, the SPME is inserted into the injection port in the same order but with duration of desorption set specifically for the GC analysis. When the SPME needle is released from the injection port of the GC, it has been completely cleaned or baked leaving no trace of the previous sampling.

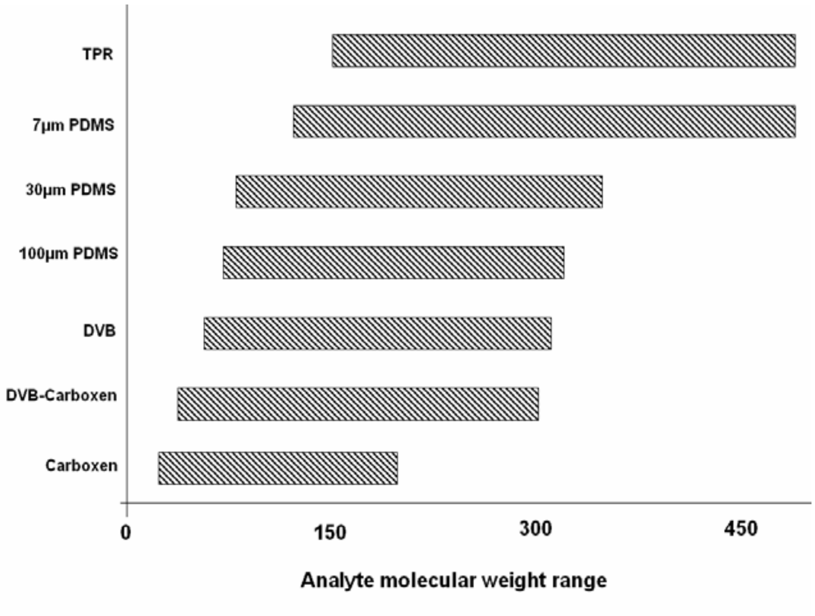


Figure. 1.3 The range of sensitivity for various SPME fibers (Sigma-Aldrich Co 2005).

1.6 Gas Chromatography-Mass Spectrometry Technique

Gas chromatography-mass spectrometry (GC-MS) is an analytical tool for separating complex mixture of chemical compounds chromatographically and analyzing its contents using its mass spectra. The mass spectrum is a unique fingerprint of a chemical compound that is obtained by fragmentation of the compound using fast electron bombardment. A very low concentration of chromatographically separated pure compound is lead into a high vacuum ionization chamber for high energy electron impact and fragmentation. The ionic fragments accelerate through a tube of charged array for analysis. Magnetic deflection influences low mass ions more than the relatively higher ones and it is used to guide ions of specific charge to mass ratio (e/m) to a collector where it is sensed and recorded. The scanning covers fragments for a programmed range of molecular weights. It is possible to use this fragmentation data and compare with pure compound data to confirm the identity of each component in a complex mixture of the analyzed sample.

The list below shows the typical steps leading to compound identification using mass spectrometry.

1. note the general appearance of the spectrum to see if it is interpretable;
2. select a candidate peak for the molecular ion (M^+);
3. examine spectrum for peak clusters of characteristic isotopic patterns;
4. test (M^+) candidate by searching for peaks corresponding to reasonable loss;
5. explore low-mass fragment ions;
6. compare spectrum to reference spectra; and
7. spectral interpretation must be necessarily reconciled with all other information about the sample.

2. OBJECTIVES

The volatiles extraction mediated by microwaves from spices would involve minimal processing and would be expected to yield superior quality of essential oils. However the process would call for optimization based on the conditions of extraction such as microwave field at the region of extraction and its influence on extraction. Such data are not reported in the literature due to the complexities of microwave process measurements.

The primary objectives of the current research are:

1. to develop a procedure for microwave extraction of volatiles from selected plant materials (black pepper and coriander) and measure the microwave field strength at the region of extraction;

2. to develop a procedure for sampling and analysis of the volatiles extracted using gas chromatography-mass spectrometry;

3. to study the effect of variation of extraction parameters such as water content and microwave field on the extraction of black pepper and coriander.

The very nature of the objectives are such that, initially every step of the research would be expected to be thoroughly examined for its intuitive appropriateness using a systems engineering approach. An optimization study would be beyond the scope of the research. However, several preliminary experiments are expected to be performed in order to arrive at a condition leading to consistently measurable values particularly for temperature rise and the GC-MS of the extract using readily available instruments and equipments. The overall aim of this research would be to bring the stated objectives together in a simple monolithic experiment placing high importance to portability and minimal cycle time for each experiment.

Towards this end, the possibility of modifying the existing laboratory microwave equipment (COBER™ electronics) would be investigated by conceiving and designing a rotary vessel inside the oven cavity for extraction. The procedures for measuring microwave heating and sensing the extraction of volatiles from black pepper and coriander powder samples would be explored. A new procedure for integrating the extraction process with process monitoring and the statistical data analysis for comparative studies will be developed.

3. LITERATURE REVIEW

The microwave extraction of plant volatiles is a relatively unexplored area of research. In the current work, it is proposed to study a procedure for microwave extraction of essential oils and compare the extraction under different conditions of water content and microwave field using SPME and GC-MS. A simple design for conducting such an integrated study is not reported in the literature. Although the science involved is basic, the daunting challenge remains in measurement and comprehending the precision and accuracy of the data obtained, taking every source of error and its magnitude into account. Data on microwave processes still remain, to a great extent, of an empirical nature. This chapter introduces the technological areas involved in the microwave processing, the empirical approaches attempted and the various challenges they pose.

3.1 Microwave Extraction

The microwave extraction of essential oils is discussed in this section, starting from the materials as well as industrial perspective of the process, based on current literature.

3.1.1 Industrial perspective

Electronic and electromagnetic technologies, that are familiar today were invented during the dismal and desperate early days of World War II. Sir Henry Tizard representing the British Forces was instrumental in setting the pace for radio detection and ranging research by the US defense, most of it at the MIT's Radiation Laboratory in 1940. The cavity magnetron revealed to the US by the Tizard mission has been described, as among the most valuable cargo to ever cross the Atlantic. The new magnetron was a breakthrough, in that it could produce microwave pulses many orders of magnitude than could anything else then in existence (Pound 1999). RADAR, NMR and microwave ovens were the outcome of the 1940s radio frequency research.

Microwave energy for heating has been in commercial use since 1950 (Edgar 2001) But it is only recently that its benefits as a environmentally-friendly source of thermal energy has been widely appreciated.

Thermal technology dictates the quality, economics and environmental impact of any processing plant. It is by far the most sensitive aspect of food processing. The rising number of Green engineering regulations (Figure 3.1) call for more efficient energy usage and more environment friendly raw materials as well as effluents (Allen 2002).

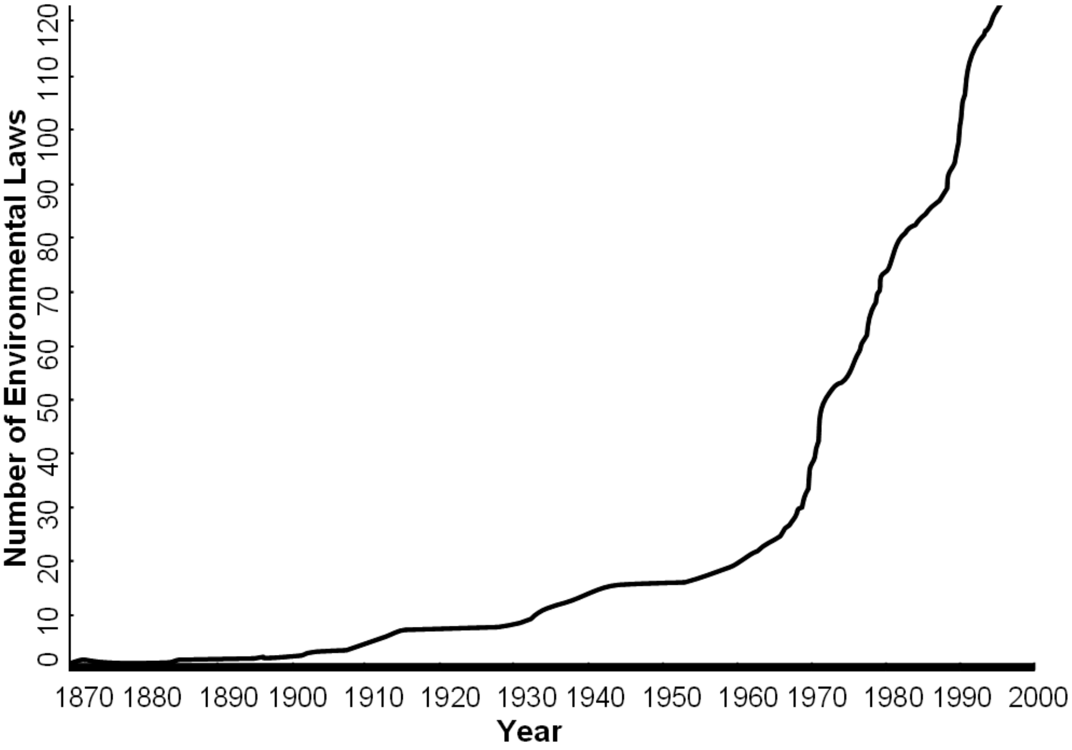


Figure. 3.1 The ever tightening environmental regulations (Allen 2002).

Efficiency demands a bare minimal processing of materials especially nutritive foods. Hence, electric heating technologies such as radio frequency, microwave, ohmic and infrared are fast emerging; among them microwave shows a highly promising future (Chang 2000). They are energy efficient and can operate in combination with other heating technologies. Table 1.1 shows a comparison of the conversion efficiency of different types of heating systems.

3.1.2 Electromagnetic radiation and microwaves

Microwaves are electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz (Singh 2001). Figure 3.2 shows the electromagnetic spectrum in terms of frequency, wavelength and photon energy; and the common names given to each region of the spectrum.

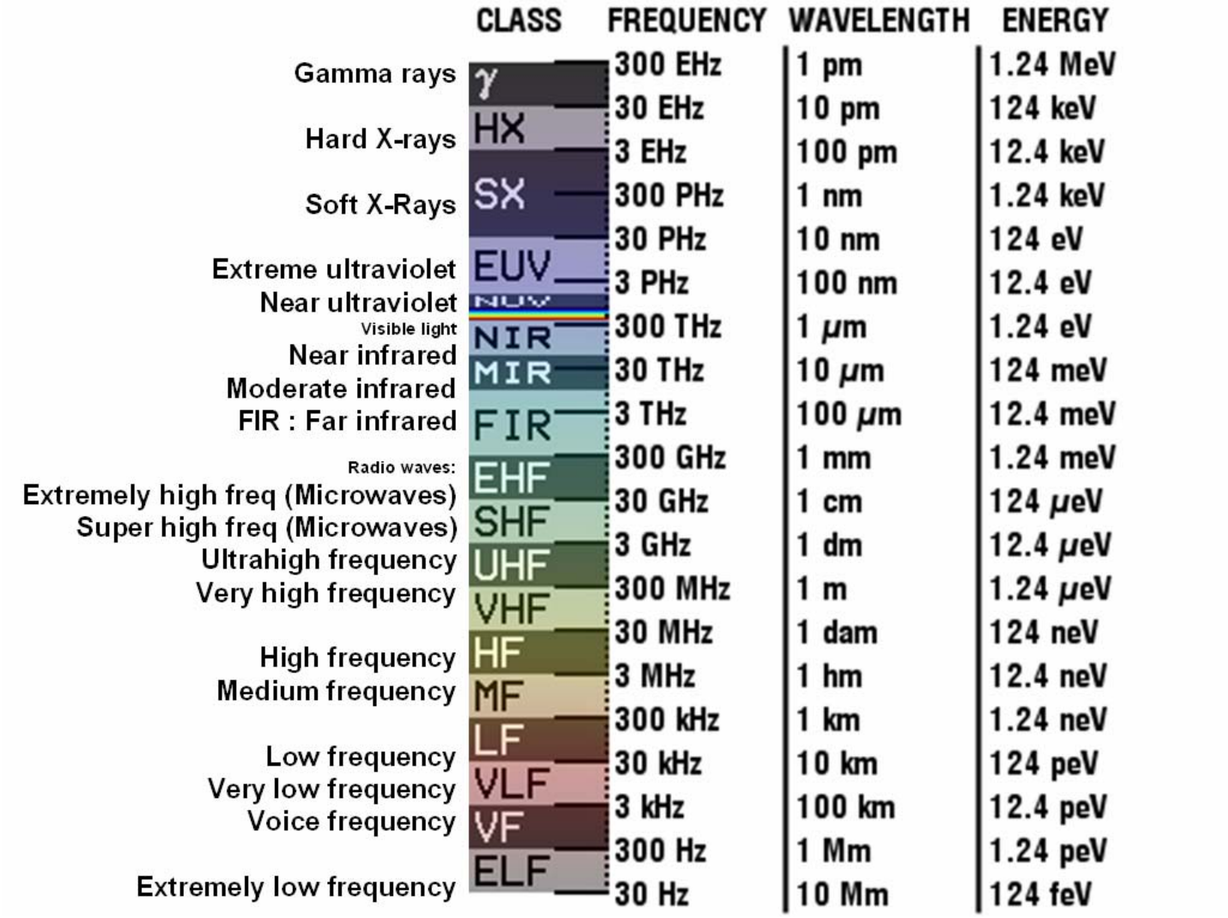


Figure 3.2 The electromagnetic spectrum (GNU 2005).

Microwave oscillations propagate with a 'time interval between peaks' ranging from 3 x 10^{-8} to 3 x 10^{-11} seconds. This range coincides with temporal sequence of events at atomic and molecular quantum transitions such as reactions in aqueous phase, molecular dissociation and most importantly (for industrial microwave technology) – dielectric relaxation in water. These atomic or molecular interactions take place between time intervals of 10^{-17} to 10^{-8} seconds. The dielectric relaxation of water may vary from frequencies of 100 MHz for bound water to 18 GHz for pure water (Asano 2003) and this is the property that is studied extensively when heating effects of microwave is investigated. The most effective conversion of microwave energy to thermal energy in biological materials (or otherwise moist materials) will occur in this frequency range.

3.2 Frequency Dependence of Dielectric Properties of Materials

The attenuation of electromagnetic waves at the microwave frequency range in a moist medium is mainly due to the interaction of water leading to orienting effects manifested as heating. This is the effect that is of interest currently. However, attenuation is caused by energy absorption at various other frequencies. The electromagnetic characteristics of materials are controlled by the macro-molecular, granular, as well as atomic scale behavior of the components constituting them. Figure 3.3 shows that the interaction of electromagnetic radiation with matter depends on the frequency as well as the material properties (Mauritz 2005). Various effects occur at different frequency creating the characteristic stepwise development of dielectric properties with respect to frequency. In this idealized representation of changes occurring over a wide range of frequencies, the relative permittivity effectively remains constant at high and low frequencies.

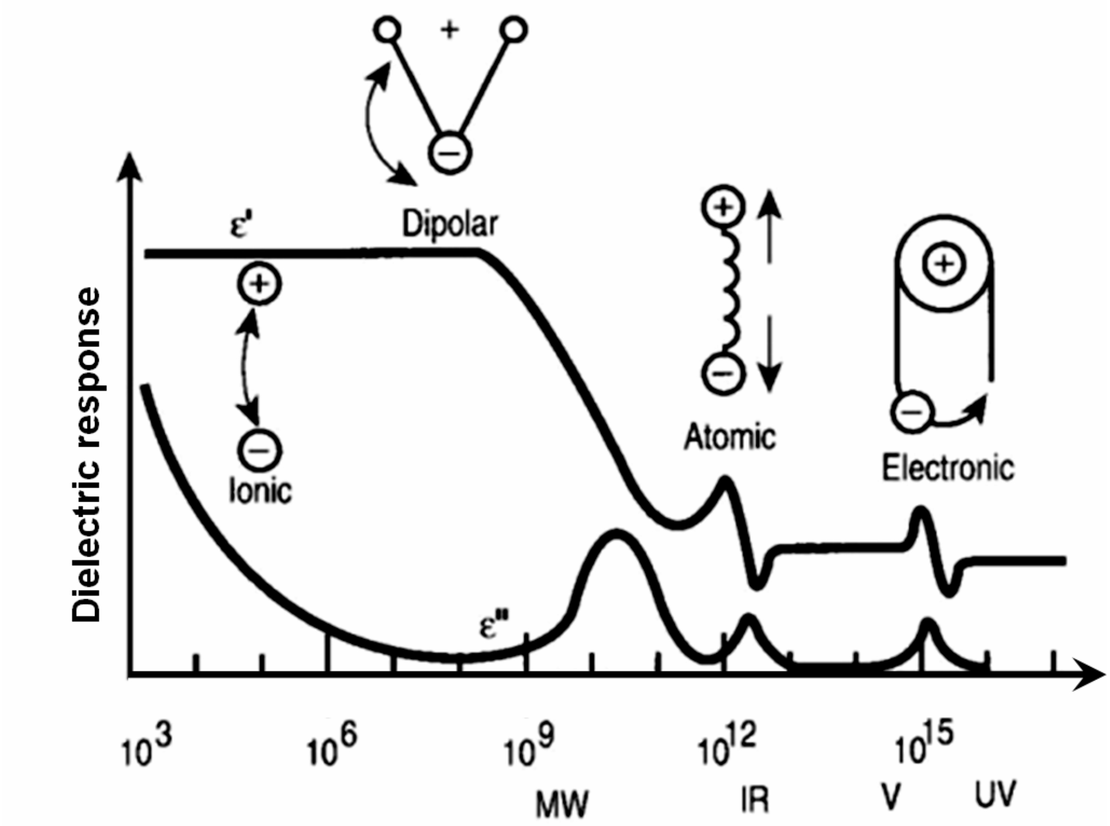


Figure 3.3 Interaction of electromagnetic radiation with matter (Mauritz 2005).

The changes occurring in the microwave region are of particular interest here. The relaxation phenomenon portrayed relates to the disturbance of polar molecules by an impressed electric field. There is an orienting effect experienced, associated with corresponding energy absorption in the quantum rotational band. If a polar molecule such as water, is present in the medium, these molecules experience a force that acts to orient the permanent dipole moment in the direction of the electromagnetic field. This force is opposed by equilibrium state of the molecules. If an alternating field continuously propagates, the individual molecules will be induced to rotate in an oscillatory manner about an axis through their centers. Inertia however prevents them from spontaneous response. Similar effects are felt in the translational degree of freedom too. The polarizing effect of propagating waves is closely related to the thermal mobility and hence, sensitive to the temperature. The temperature, activation energy and the natural frequency of oscillation of polarized particles determine the relaxation time, often expressed as relaxation frequency (Lew 2002).

The relaxation frequencies of materials vary widely and even vary between the same molecules in different phases (Meredith 1998). Ice, for instance has maximum absorption at 10^3 Hz, whereas, it takes place in the region of 10^6-10^{10} Hz in water. There are various influences that are relevant in studying relaxation such as polarizing mechanisms associated with ionic atmospheres surrounding the particulates, interfacial phenomena, adsorbed water and pore effects.

Water shows the highest response to the microwave interaction. Water has a relative permittivity of 80, whereas porcelain has about 5 at microwave frequency and room temperature. The significantly selective microwave interaction of water and the resulting thermal conversion forms the basis for agro-industrial microwave processes. However, between water and porcelain, there is a whole range of substances that heat in the presence of microwaves (lipids are notable among them). In the current experiment, the use of spice powders having low water content (~10%) is proposed as microwave substrates.

3.3 Industrial Application of Microwave Processing

International convention dictates that microwave ovens (and other Industrial, scientific and medical microwave applications) operate at specific frequencies; the most favored being 2.45 GHz. Raytheon Company, MA, USA under Percy Spencer was an early leader in the development of domestic microwave oven. It was Percy who chose 2450 MHz as the suitable frequency (Edgar 2001). At this frequency, the electric field swings the orientation of water molecules 2.45 x 10^9 times every second and the chaos inherent to the system opposes the synchrony of the oscillation with that of the field. Thus creating an intense heat that can escalate as quickly as several degrees per second (estimated as 10^0C/s at 4.9 GHz) (Lew 2002).

Water is the predominant component of biological materials, its presence directly influences heating. However there are minor contributions from a host of other factors (Shiffman 1986, Kaufmann 2002): heating is accelerated by ionic effects (caused by salt content) and specific heat of the composite material (Decareau 1992). For instance in oily materials, the influence of specific heat becomes the determining factor in microwave heating owing to low specific heat of oils, often less than half that of water (Ohlsson 1983). The dielectric constant is a good indicator of the extent to which the heating can be achieved using microwaves (Kaufmann 2002). Dielectric constants of various solvents are shown in Table 3.1. From this table, the reason for choice of aqueous or polar phase as the favored medium for microwave process can be appreciated. Water and methanol heat rapidly due to their high dielectric constants. Water has the advantage in that it boils without explosiveness or inflammability.

Table 3.1 The dielectric constant values of various solvents (Kaufmann 2002).

Solvent	Dielectric Constant (20^0C)	Dipole moment (20^0C) (Debye)
Hexane	1.89	0.1
Tolune	2.4	0.36
Dichloromethane	8.9	1.14
Acetone	20.7	2.69
Ethanol	24.3	1.69
Methanol	32.6	2.87
Water	78.5	1.87

Microwave ovens come in a variety of designs. However, the underlying principles of operation are very much the same. Microwaves are generated inside an oven by the alternating current from domestic power lines at frequency of 60 Hz and stepped up to 2450 million Hz. This is accomplished by a device called magnetron which operates at 4000 to 6000 volts inside microwave ovens. The step up transformer that powers the magnetron accounts for more than half the weight and value of the domestic microwave ovens. A waveguide channels the electromagnetic waves through a conduit called waveguide, into the cavity that holds samples/substrates for heating. Domestic microwave ovens have highly reflecting cavity walls and a rotating reflector/fan that produce several modes of microwaves maximizing the efficiency of heating. However in some chemical researches where temperature is closely controlled, it has been considered desirable to use single mode ovens that homogenously distribute the microwaves into the reactor. The nature of two modes in operation is shown in Figure 3.4.

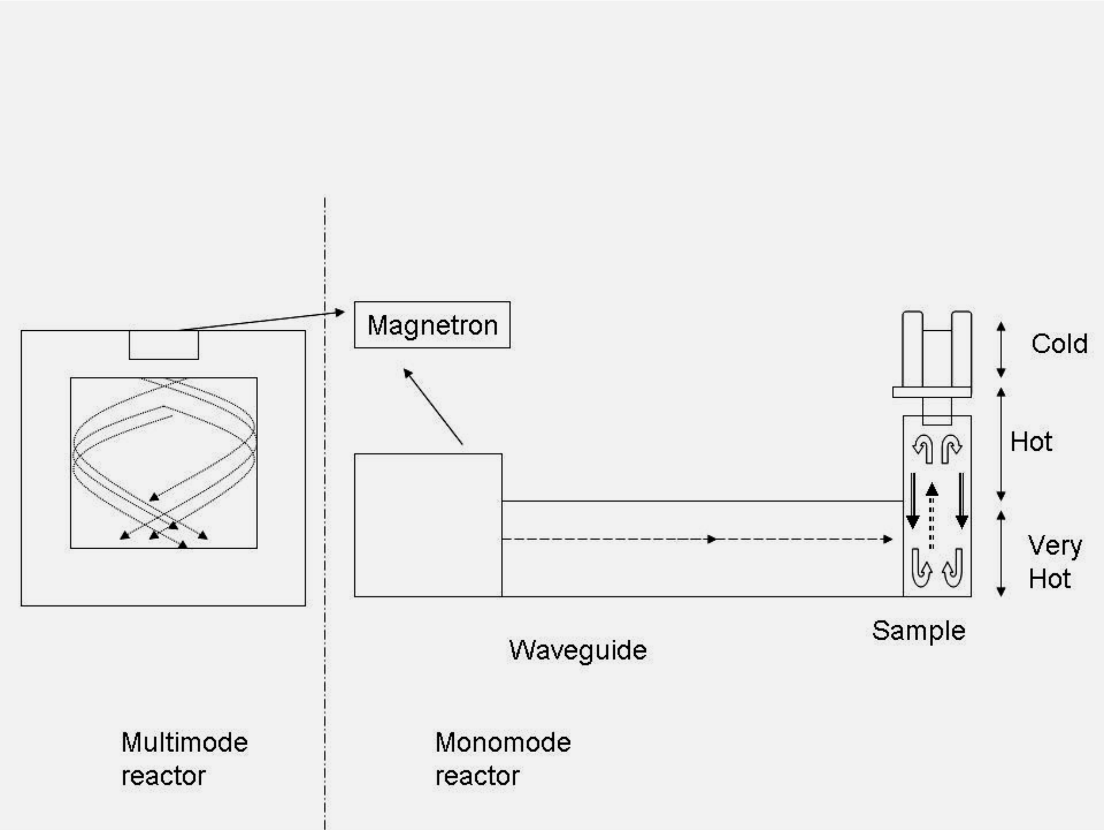


Figure. 3.4 Monomode and multimode ovens (Lew 2002).

Over 60% of homes in the UK and about 90% of homes in US have a microwave oven and an ever-increasing range of foods is produced with microwave reheating instructions. Although, the potential industrial applications of microwaves are innumerable viz. processing of food, rubber, textiles, wood products, ceramics, waste etc., the uptake of microwave processing by industry has been far less dramatic. (Giese 1992, James 2000). Areas that have shown promise and where systems have been developed are drying, cooking of patties, pasteurization of ready-to-eat meals and the tempering of meat and butter. There is however a growing body of work on various new applications. The following are some of the applications: packaging (Andreasen 1988, Turpin 1978); defrosting (Benzanson 1973, Cathcart 1946, Martin 1945); browning (Brastad 1980, 1981, Turpin 1989); microbial inactivation (Mallikarjunan 1994); tempering (Meisel 1972); dehydration (Kiranoudis 1997); blanching (Wrolstad 1980) and aroma extraction (Ramanadhan 2003).

3.4 Essential Oils

Essential oils are the volatile fraction of the secondary metabolites produced by plants. Essential oil bearing plants have been valued historically for their medicinal, culinary and fragrance properties (Parry 1969). Most commercially exploited essential oils are monoterpenes. However, the essential oils are highly complex and may include oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetals, ethers and esters) apart from pure hydrocarbons; and also at times, trace of potentially harmful nitrogen containing compounds (such as alkylpyrazines and akylmethoxypyrazines found in coriander) (Schnaubelt 1999). The spices, it must be admitted, have been in use since time immemorial and it has only recently gained in acceptance and reliability as an important source of bioactive compounds. The term bioactive compounds is not very easy to define and its purpose of production by plants still remains to be established completely.

But in general terms, bioactive compounds can be understood as follows. We normally monitor our diet in terms of its nutritional value, i.e., its contribution to our sustenance and growth. Recently scientists from disciplines such as neurology and immunology started to look for deeper answers from human diet. When human elements of our diet serve beyond the basic nutrition and contribute to enhancing immune or mental health, these elements are referred to as bioactive elements of human diet. Many examples of such elements can be found in traditional diets, most outstanding of which are found in the essential oils of spices and condiments.

"For an essential oil to be a true essential oil, it must be isolated by physical means only. The physical methods used are distillation (steam, steam/water and water) or expression (also known as cold pressing, a unique feature for citrus peel oils). There is one other method of oil isolation specific to a very limited number of essential oil plants. This is a maceration/distillation. In this process, the plant material is macerated in warm water to release the enzyme-bound essential oil. Examples of oils produced by maceration are onion, garlic, wintergreen, bitter almond, etc. What is not an essential oil is a CO_2 extract, a halohydrocarbon extract or an empyreumatic distillate" (NAHA 2005).

Figure 3.5 shows the basic natural volatile extraction flow sheets of different conventional operations necessary to obtain an essential oil or derivative (Weiss 1997). There are several procedures well established for the extraction of the flavor, fragrance or medicinal aroma. The most delicate fragrance and flavour components from flowers are extracted by concentration using solvent wash. Repeated wash of the delicate flowers with a solvent such as hexane, gives a broth concentrated with aroma components called concrete. Concretes are purified to yield essential oils. The identification of all the compounds in a species as well as scanning for valuable volatiles in all aromatic plants is still far from complete. Three areas that have severe shortcomings are:

(1) The postharvest handling of aromatic plants. Usually raw materials are not extracted within the farm gates due to sheer logistic reasons and also due to the lack of portable essential oil extractors.

(2) The classification of species in terms of essential oil composition. Oil composition, especially the terpene components, is now important in plant classification as chemotaxonomy, since physical characteristics of plants may virtually be impossible to differentiate finely (Weiss 1997).

(3) Screening for new high value aromatic plants. Only 1.7% of aromatic plants are used as raw material sources for the flavor and fragrances industries worldwide. Of these, 50% are obtained from the wild (wild crafted). Only about 1% of the aromatic plants are cultivated (Lawrence 2002). Yet essential oil is a multibillion dollar industry. Flavors and fragrances is forecast to grow over 5.0 percent per annum (Freedonia 2003). The industry has potential for enormous growth.

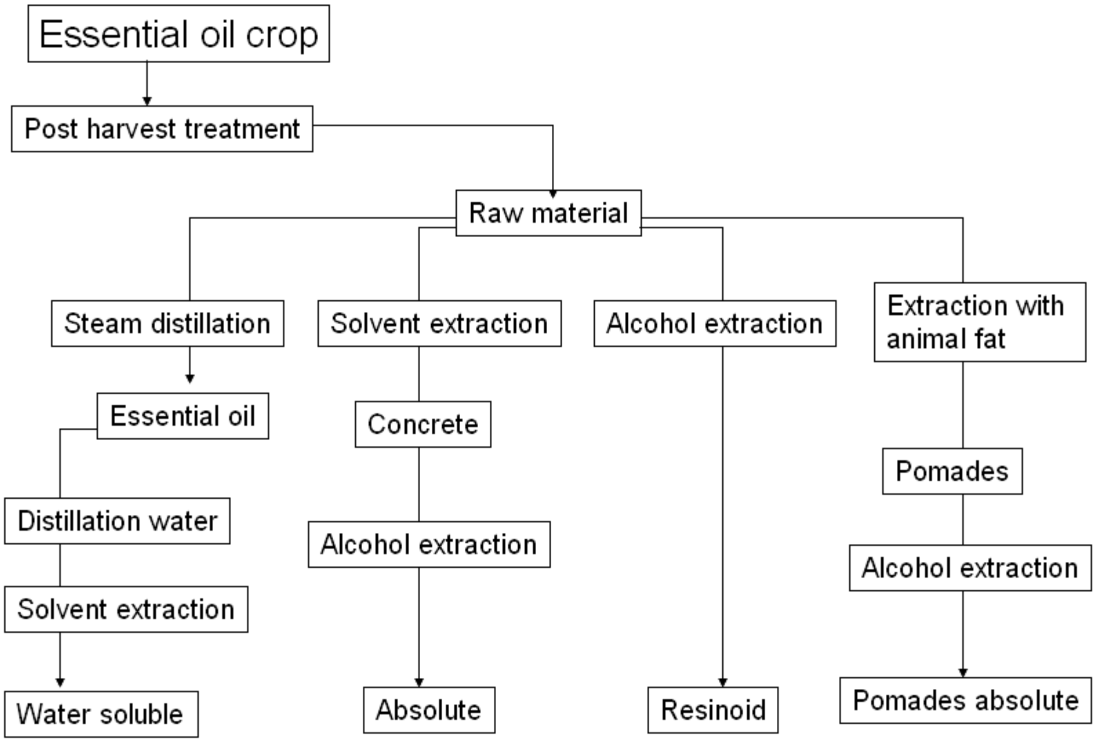


Figure 3.5 The basic natural volatile extraction flow sheets.

In the current research, two aromatic species have been chosen to study a procedure of extraction and analysis of essential oils in a Green process that is rapid, portable and does not use organic solvents. This process may contribute to the three areas stated above namely chemotaxonomy, in situ extraction and high throughput screening.

3.5 Pepper and Coriander

The botanical species selected for the current research viz. pepper, and coriander fall under the category of spices, by far the oldest botanicals to be traded (Uni-Graz 2005). Since 3000 BC, spices have been traded (detailed in recorded history of the ancient Sumerians). Spice demand has never declined since. In 1498 the Portuguese arrived in the east coast of India. Their ships carried 3300 tonnes of spices, mainly pepper, to Lisbon annually. Today about 700,000 tonnes of spice move in the international trade, estimated at roughly $2 billion for The European Union alone. The United States imports around $1 billion value of spices (CosmeticIndex 2001). Spices are being used in culinary as well as medicinal ingredients in the east and recently the trend has picked up in the west, thanks to the herbal revolution of the 90's as well as booming oriental cuisine market. Spices are categorized rather loosely on the basis of their organoleptic properties. Spices are defined as dried parts from the aromatic plants. From aromatic plants, emanate volatile essential oils that have been used as flavors, fragrances and medicinal aroma. With industrialization and burgeoning customer demand, there has been a heightened consciousness about the inadequacy of prevalent processing technologies. The market for the spices are divided equally among three sectors namely: manufacturing, catering and retail. Quality and consistency have been, for long, the two elusive goals of the suppliers.

The global fragrance market where monoterpenes play a major role, is booming. Over the last five years, the market has grown at an average annual rate of 2.3%, and in 2000, was valued at $23.3 billion. (CosmeticIndex 2001). Yet, to this day aromatic plants are distilled the same way our forefathers did several thousand years ago. Essential oils are predominantly obtained by steam or hydro distillation. Various other technologies have been tried but have focused primarily on sampling (Zorgati 2000) or extraction of low volume special natural products.

Overall, the botanical species and their extracts are traded at close to a value of hundred billion dollars. This is a significant market, worthy of being subjected to intensive species wise research. The current research chooses two of the most significant botanical species viz. coriander and black pepper. Pepper and coriander happen to be very important spices used in a great variety of foods and beverages across the world. In terms of quantity, black pepper is the highest traded spice in the world. The chemical composition of these two spices, is given in Table 3.2.

Table 3.2 Composition of black pepper and coriander (Pruthi 1962).

Spice	Water content (% w.b)	Volatile oil (% ether extract)	Ash (%)	Alcohol extract (%)
Coriander seed	7.52	0.3	4.31	22.16
Pepper black	7.5	3.10	0.08	10.8

3.5.1 Black Pepper

Black pepper or *Piper nigrum* L. is a corn that grows as drupes in pepper shrubs. A brief description of this spice is given in Table 3.2, Table 3.3 and Figure 3.6 shows the cross-section of pepper corn. The main components of pepper essential oil are sabinene (3.9-18.8%), β-pinene (3.9-10.9%), limonene (8.3-19.8%) and β-caryophyllene (28.4-32.9%) (Uni-Graz 2005; Pruthi 1962). Although black pepper is the most popular form of pepper spice, there are also the white, green, red and rose peppers that have marginal commercial value. The pungency of the black pepper comes from the outer black skin consisting of the pericarp. Figure 3.6 shows a pepper fruit/corn. The pepper fruit is shown here with exaggerated features of its insection to visualize the interior in detail. The oil bearing idioblast cells are found in the perisperm. The volatile oil which gives the characteristic aroma of pepper is present in the inner white endocarp. Pepper postharvest processing consists of enzymatic blackening, drying, pulverizing and oleoresin extraction combined with limited stripping for volatiles.

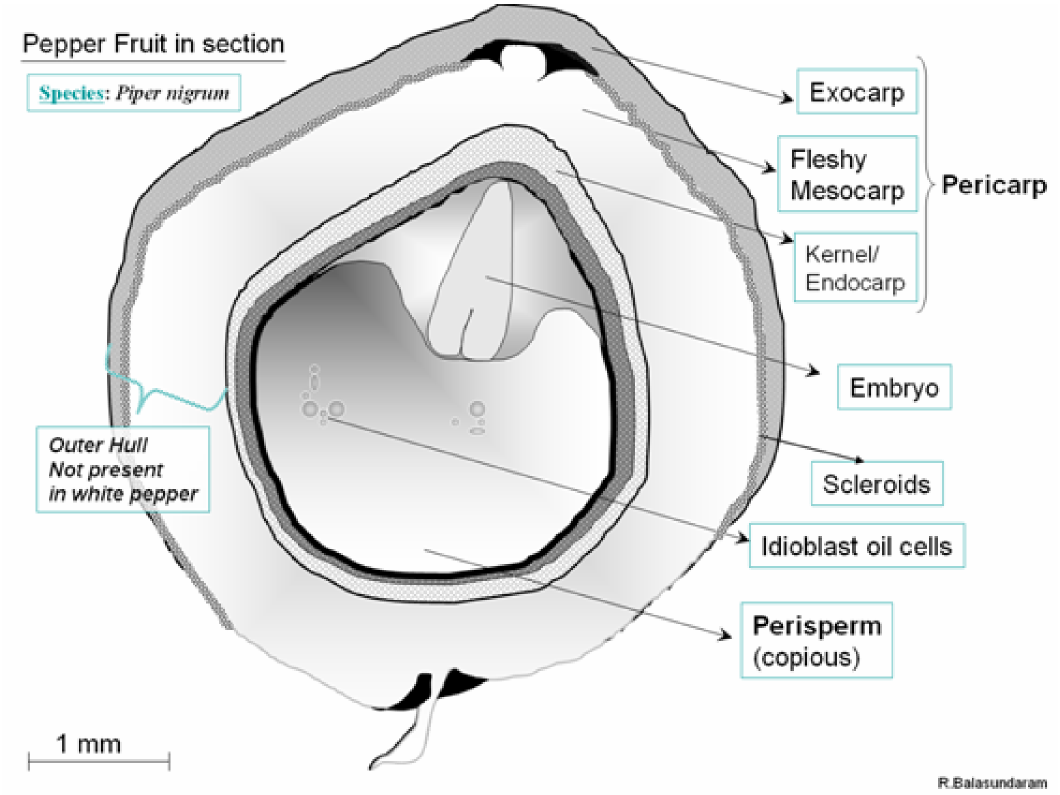


Figure.3.6 The pepper fruit.

There is a significant loss of valuable components by the time pepper arrives to retail outlets. The processing operations right from harvest stage through a storage and unit operations involving heat such as drying and pulverizing lead to losses. When losses are minor, often the marketed spices are enhanced or spiked with specific pure compounds either synthetic or natural. Hence what is seen here is a need for: (i) processing that can be carried out within farm gate considerably precluding storage and transport; (ii) portable analytical scale extractor that rapidly extracts volatiles for quality testing; and (iii) a procedure for sampling volatiles in situ with enough stability of sample storage for transport and later analysis.

Table 3.3 Botanical features of black pepper and coriander.

Name	Botanical Name	Family	Type	Valuable Part
Black Pepper	*Piper nigrum* Linn.	Piperaceae	Vine, Perennial	Fruit/Berry
Coriander	*Coriandrum sativum* Linn.	Umbelliferae	herb, annual	Fruit/Seed, leaf

With this enhancement of the value chain, the quality and economy of the spice trade would immensely improve. A survey has shown that sensory quality ranks higher than nutritional value and price (FMI 1993). The sensory perception (odor, taste, color and texture) are highly reflective of an expeditious postharvest operation.

3.5.2 Coriander

Tables 3.2 and 3.3 respectively give the important chemical and botanical features of coriander. Coriander comes from the carrot family. In terms of aroma, this family ranks equivalent to mint, rosemary, thyme, marjoram, oregano, basil and sage. All the parts namely leaves, roots, fruits and seed of this family have high value. Figure 3.7 shows a coriander seed and its cross-section. What is usually called the seed is in fact the fruit with seed inside. The oil-bearing idioblasts are present in the boundary of fruit and seed marked in the Figure 3.7 as oil glands.

Coriander leaves have gained a very high popularity in the American food industry owing to the high demand for Mexican dishes such as salas. Although coriander spice usually is marketed as seeds, they are partly fruits with the seed within. Just as in pepper, the wall or skin of the fruit contains rich flavor compounds. However, unlike pepper, the aroma or the volatile components are also peripherally located and what is extracted for fragrance, flavors and medicinal aroma comes mainly from the outer layer of the seed.

The main compounds present in coriander are camphor, geraniol (acetate), linalool and enantiomers, α-pinene, γ-terpiene and *p*-cymene (Uni-Graz 2005). The monoterpenes such as linalool which are predominant component of coriander essential oil are volatile and are considerably lost during postharvest processing. Linalool also has significant medicinal application as it has been found to be effective against a range of gram-positive and gram-negative bacteria as well as yeast and molds (Wan 1998). Considering the value of the volatile components not only in terms of aroma but also in terms of bio-activity and medicinal applications in particular, processing facilities that are portable to be used within the farm could make significant difference as previously pointed in the case of pepper extraction.

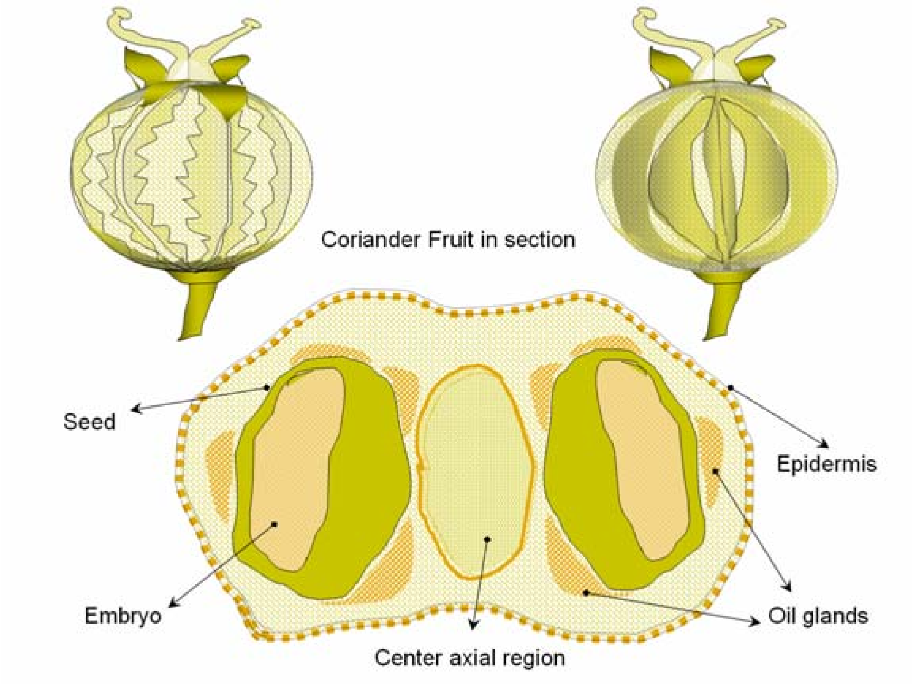


Figure 3.7 Coriander fruit and seeds.

3.6 Design Criteria for Microwave Extraction

In section 1.4a it was seen that an efficient processing (of powders for example, as in the current research) involves:

1. uniform distribution of material exposed to the microwave and
2. uniform irradiation of microwaves upon all the particles constituting the bulk.

Several approaches are taken to achieve this as seen in the various models of microwave extractors reported in the literature. There are many manufacturers that have teamed up with research groups to constantly innovate microwave processes; three major manufacturers among them being CEM Corp. from the United States, Milestone Inc. which is an Italian-based company and Personal Chemistry AB (known as Biotage since 2003) from Sweden(CEM 2000, Milestone 2005, Biotage/Personal Chemistry 2005). The recent microwave extractors are closely systems-engineered and come with features for expandability, portability and compatibility with various data analytical options (CEM 2005; Milestone 2005; Biotage 2005). They essentially seem to have one common goal, and that is to address analytical scale extraction, which, to an extent, precludes the necessity to design scale-up possibilities. It is important to note that essential oils are mostly high-volume processes although the analytical scale is also an equally vital operational area. The current research attempted the development of a procedure applicable to analytical scale with inherent scale-up possibilities for industrial applications based on the two points introduced in section 1.4a. Before delving further into extraction techniques, it is important to note that microwave studies lead to various other applications, left to the ingenuity of the technologists, since the underlying microwave principles and findings are pervasively applicable.

3.6.1 Application of microwave mediated methods

Microwave application in agricultural and food technology is a fast growth industry. It is increasingly finding applications in tempering, vacuum drying, freeze drying, dehydration, cooking, blanching, baking, roasting rendering, pasteurization, sterilization and extraction (Lew 2002; Wathey 2002; Lidstrom 2001; Larhed 2001 Ramanadhan 2003). The utilization of microwaves in applied natural product areas such as pharmaceutical and analytical chemistry as well as flavor and fragrances industry has just begun in recent years. The detailed heat transfer characteristics in a microwave cavity have only in recent years, become amenable to mathematical modeling (Yakovlev 1999) following rapid advances in information technology. The same applies to comminution, maceration and bulk-solids process modeling. Traditionally, plant materials were subjected to mechanical shear to release the volatiles in virgin state. Many proposed microwave-based methods often adopt comminution for pre-treatment (Raman, 2002). In industrial scale, annatto (that which gives cheddar cheese its golden hue), for instance, is extracted by particle attrition and impact using spouted bed and a ball mill (Mendonça 2001). From macerated species, there have been attempts to use solvents to effect microwave extraction (Nélida 1999; Raman 2002). The environmental concerns with solvents is well documented in the current literature, and that is not where the environmentally sensitive technological future is heading. Microwave processing has gained acceptance as a mild and controllable processing tool. Microwave extraction allows simple, rapid and low solvent consuming processes. Ideally microwave extraction would have to combine with drying and grinding stages soon after harvest. In the current design discussed in chapter 4, such a possibility has been included.

3.6.2 Prevalent microwave extraction models

There are many microwave extraction methods suggested by manufacturers. Diagrammatic representation of seven reported models is given in Figure 3.8a to 3.8h in the order given below:

a) CEM solvent extractor;
b) microwave assisted extractor;
c) microwave reflux;
d) Sub-500 W microwave extractor;

e) drydist model of milestone;
f) solvent free extractor;
g) Monolithic equipment for microwave assisted extraction; and
h) Closed vessel mono-model of CEM Co.

The solvent extractor (Figure 3.8a) is similar to (Figure 3.8h), both are CEM systems. In order to overcome the non-homogeneity of the field, the cells are placed on a rotating carousel as in a domestic oven (CEM 2000). Going by similarities, all the extractors shown above are identical in one respect and that is the existence of segregation of particulates (for powder raw materials) with a potential for uneven absorption of microwaves.

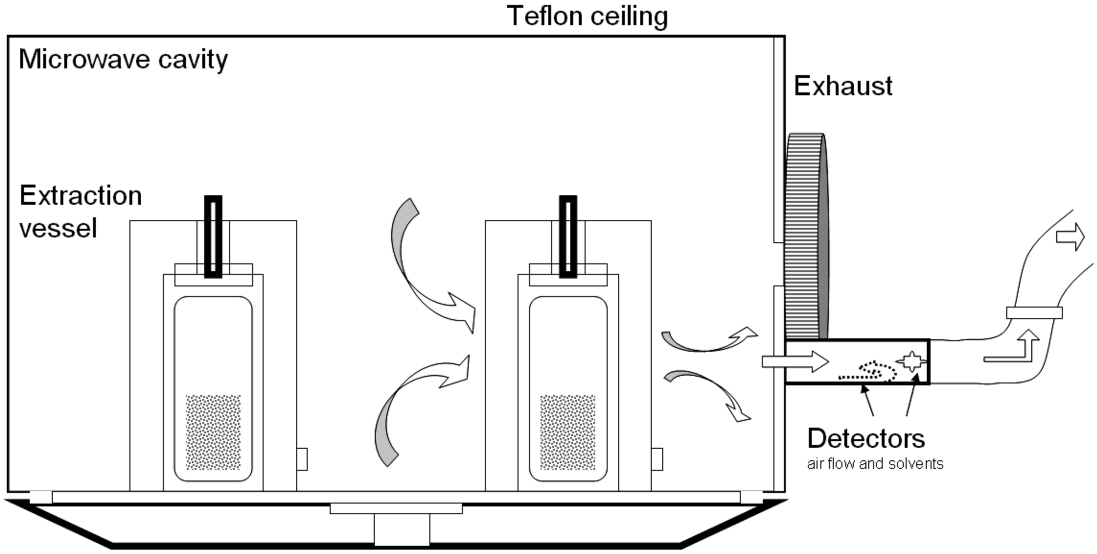


Figure 3.8a CEM Solvent Extractor: Microwave-Accelerated Reaction System, Model MARS – X (CEM 2000).

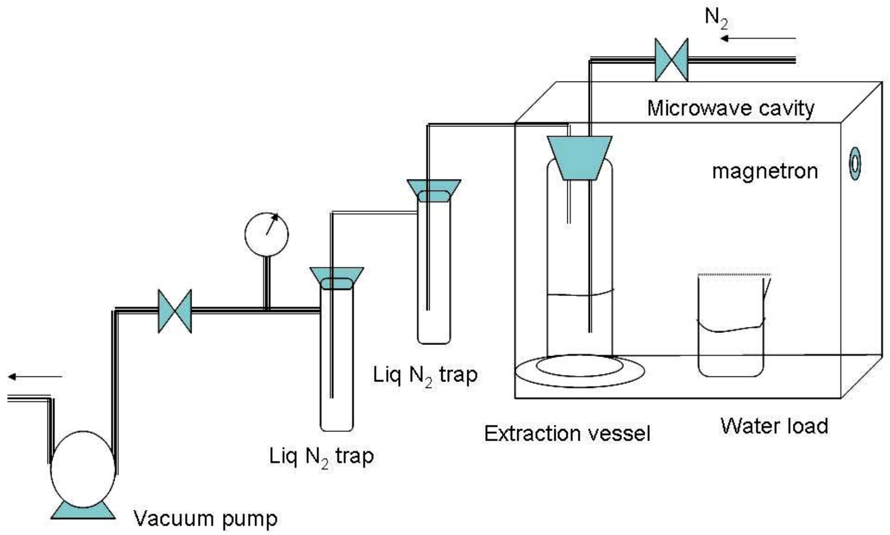


Figure 3.8b Microwave assisted extraction of black pepper (Raman 2002).

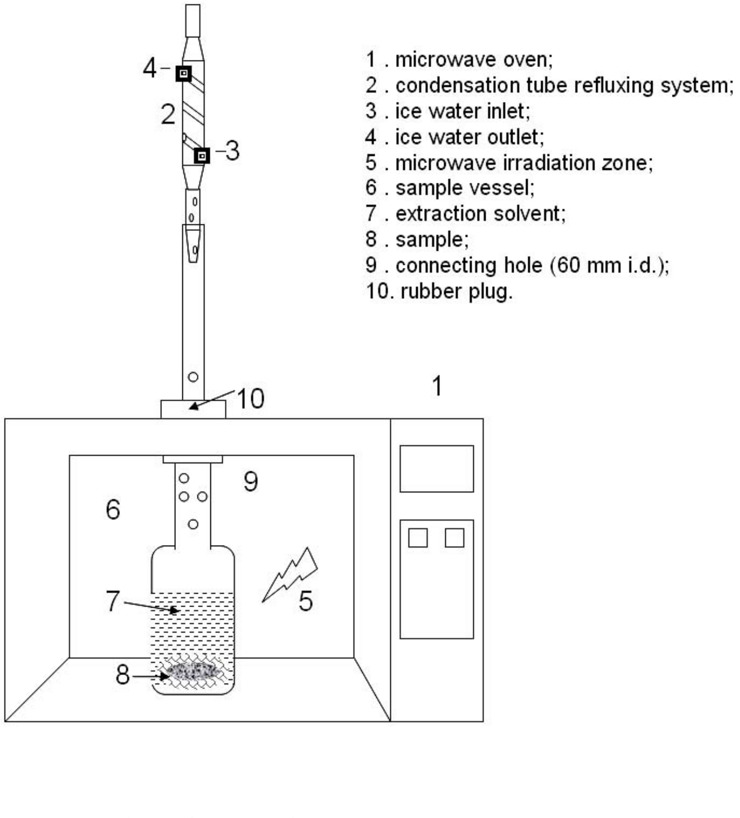


Figure 3.8c Microwave reflux (Li 2004).

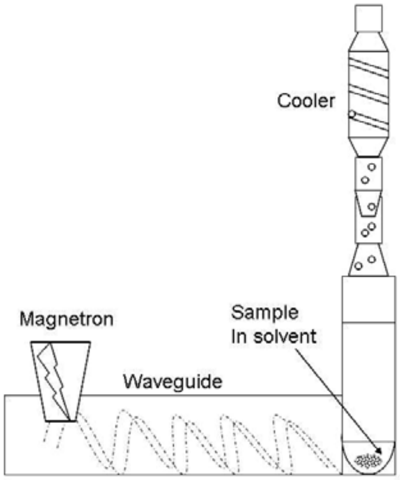


Figure 3.8d Sub-500 W microwave extractor.

This operates at atmospheric pressure and takes large samples. The open cells are made of quartz topped by a condenser. (Kaufmann 2002).

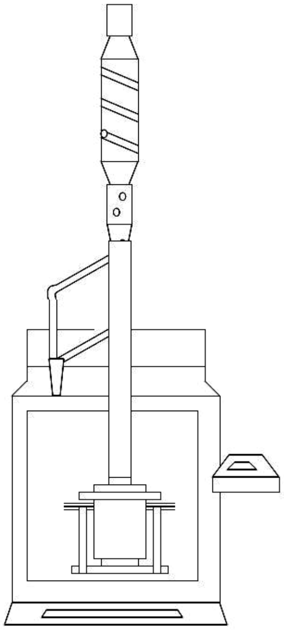


Figure 3.8e Drydist model for hydro distillation of essential oils (Milestone 2005).

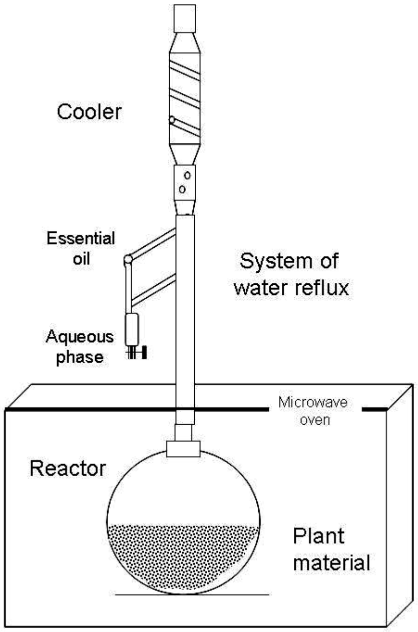


Figure 3.8f Solvent free extractor (Lucchesi 2004).

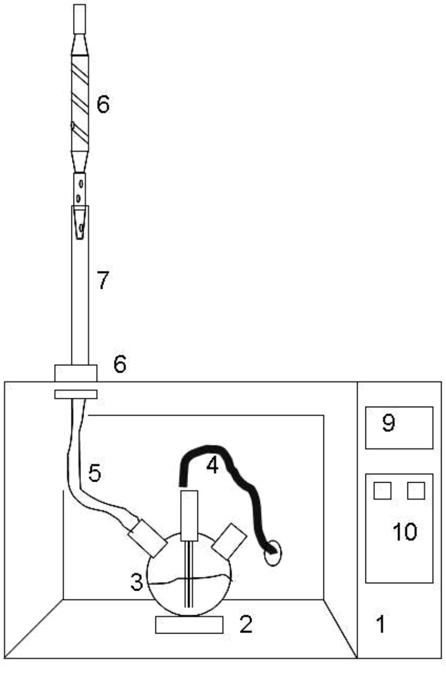


Figure 3.8g Batch equipment for Microwave Assisted Extraction (Gao 2004).

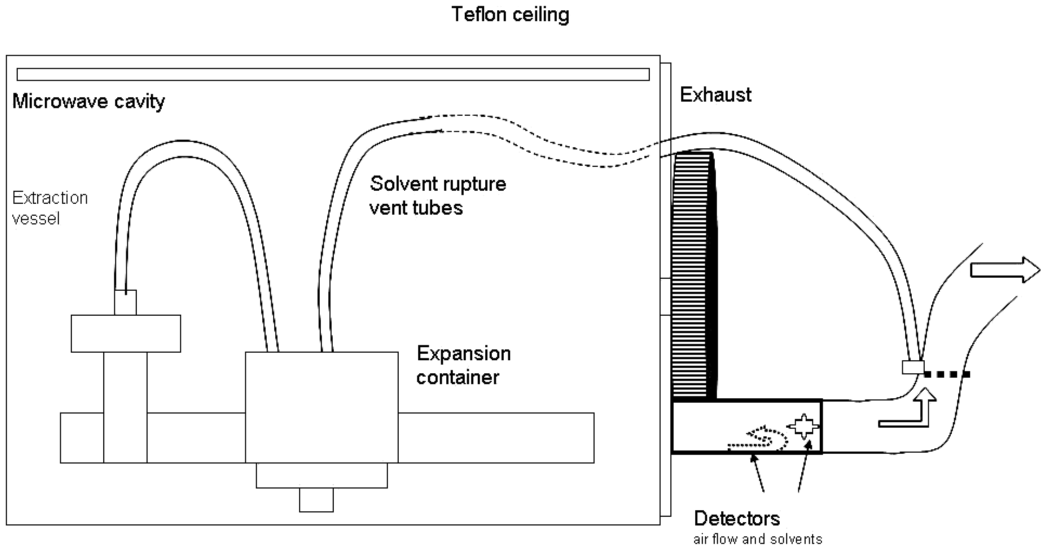


Figure 3.8h A schematic diagram of a closed vessel model from CEM Co. (Kaufmann 2002).

The literature reference on the various models shown above has been included in parethesis along with their description and its elaborate details are out of the scope of this section. However the salient point to be noted is that if the models are scaled-up for industrial application (which, however might not be the stated purpose of these designs), it is highly likely that they would experience thermal gradient and non-uniform microwave exposure of various regions of the sample as visualized in Figure 3.9. It is intuitively difficult to expect a reliable scale-up of microwave extraction that can get attenuated significantly before it reaches the sample boundaries.

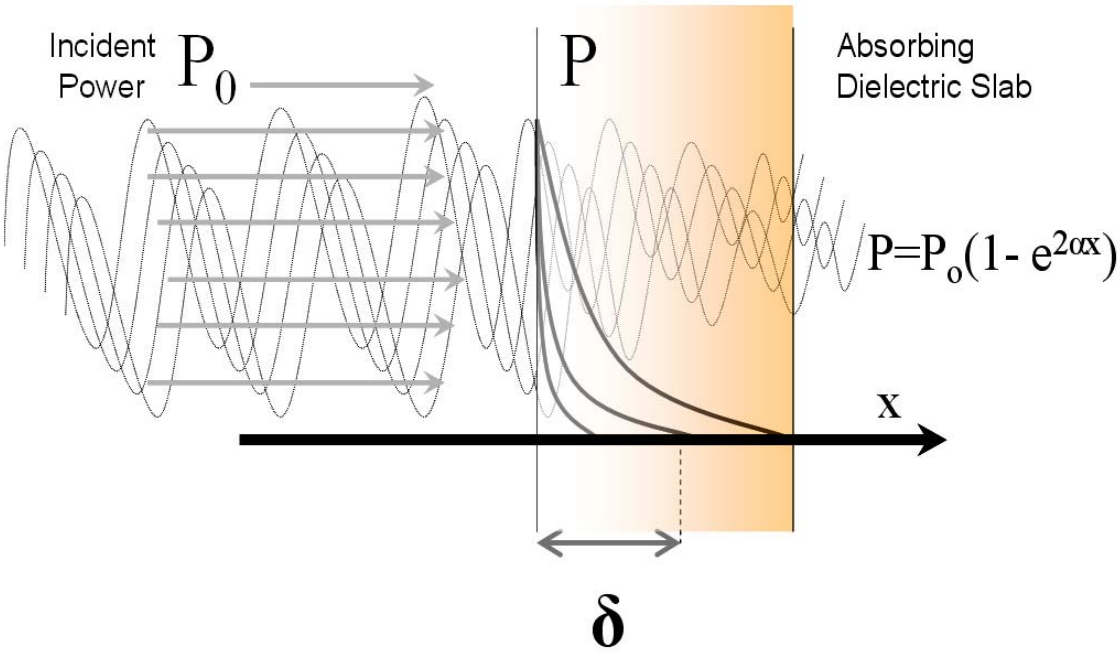


Figure 3.9 Microwave attenuation inside a sample.

The various methods of extraction that are not covered here, such as sonication, super/sub-critical fluid extraction etc have extraction capabilities and limitations owing to their inherent and unique features but they have not been comprehensively covered in the literature surveyed during the current research because their principle of operation is far removed from the method designed for the current experiments.

It is worth pointing out that the Drydist extractor in Figure 3.8e is the diagram of a mini extractor that has applications similar to the extractor designed in the current research (except that Drydist is a static model). A combination of features present in the Drydist and the model presented in the current research is expected to yield highly fruitful results. Drydist or MAHD (microwave assisted hydro-distillation) method has been compared in Table 3.4 with other extraction methods to show similarities in results for rosemary (Presti 2005). The methods compared are solvent extraction (SE), microwave assisted hydro-distillation (MAHD), hydro-distillation(HD), supercritical fluid extraction (SFE). If the benefits of using microwaves in terms of time, man-power training and environmental impact are included, the MAHD would stand apart as a more desirable than SE, SFE, HD.

Table 3.4 Comparison of various techniques for essential oil extraction.

Compound	Literature*	SE*	MAHD*	HD*	SFE*
alpha pinene	9.0-14.0	12.1	8.1	8.6	2.3
camphene	2.5-6.0	3.5	2.7	2.6	1.1
beta-pinene	4.0-9.0	4.5	6.3	4.8	2.3
myrcene	1.2-2.0	1.9	2.1	2	1.1
p-cymene	0.8-2.5	-	0.5	1.6	0.7
1,8 ceneole	38.0-55.0	50.8	45.8	56.9	35.6
camphor	5.0-15.0	2.5	5.9	5.9	3.7
borneol	1.5-5.0	2.8	2.9	2.5	6.8
alpha terpineol	1.0-2.6	3.3	3.1	2.3	5.9
bornyl acetate	0.1-1.5	0.6	1.1	0.5	1.1

SE :solvent extraction; MAHD :microwave assisted hydro-distillation; HD : hydro-distillation; SFE :supercritical fluid extraction. * values representing comparative ratios.

Microwave extraction methods are hence gaining greater acceptability in sensitive processes especially essential oil extraction. There has been an increased preference during recent years to such methods in the published literature.

The more the prevalent microwave extraction models are studied, the more convincing is the fact that substrate distribution and randomization are keys to the efficiency of microwave extraction. This formed the basis of a novel design that was started in August 2003 towards the current research.

In the current design spice powder is rotated in a glass ampoule inside a microwave oven so as to avoid particulate segregation. The glass ampoule is sealed with a gas tight septum that permits sampling using a polymer tipped syringe using a technique called solid phase micro extraction (SPME). The design makes essential oil extraction quick and portable for analytical purposes and scalable for industrial applications.

3.7 Sampling and Analysis of the Extracted Volatiles

The selection of the appropriate polymer needle is vital to the SPME procedure. The SPME has been used for adsorption of different range of molecular weights or sizes of molecules. It is important to appreciate the fact that the needle in the current research is used for sampling monoterpenes, followed by transport and injection of the extracted essential oil into GC-MS for comparative study. The fibers are chosen based on polarity as well as molecular weight sensitivity. Table 3.5 gives a listing of the fibers (Sigma-Aldrich 2005) used in SPME based on its polarity.

Table 3.5 SPME Fibre Categories (Sigma-Aldrich 2005).

SI No	Polarity of the SPME fibres
	Nonpolar Fibres
1	Polydimethylsiloxane (PDMS): 100 μm, 30 μm, 7 μm
2	**Polar Fibres**
	85 μm Polyacrylate
	65 μm Carbowax ® - divenyl benzene Stableflex ^{TM} (CW-DVB)
	50 μm CW-templated resin (CW-TPR) (HPLC only-crimped)
3	**Bipolar Fibres**
	65 μm PDMS-DVB StableFlex
	75 μm Carboxen TM –PDMS StableFlex
	55/30 μm DVB-Carboxen-PDMS StableFlex
	60 μm PDMS-DVB (HPLC only-crimped)

The current research used a bi-polar fiber called carboxen. Carboxen is useful in the volatile range of the essential oils that is extracted in GC-MS analysis mostly comprising of sub 150 amu terpenes (predominantly monoterpenes).

The SPME technique used in conjunction with GC-MS has been widely reported (Wardencki 2004). The GC-MS is a fairly standard instrument for volatiles analysis. The instrument has a broad capability and needs to be calibrated to give specific information that is of relevance to the experiment conducted.

The Figure 3.10 is a schematic diagram of the mass spectrometer, consisting of fragmentation of separated component of a mixture and its detection at the collector. The MS process involved in identification is simple to visualize; it is also portable and highly reliable, having helped in opening up new areas of research serving as a powerful discovery tool (Wisc 2005).

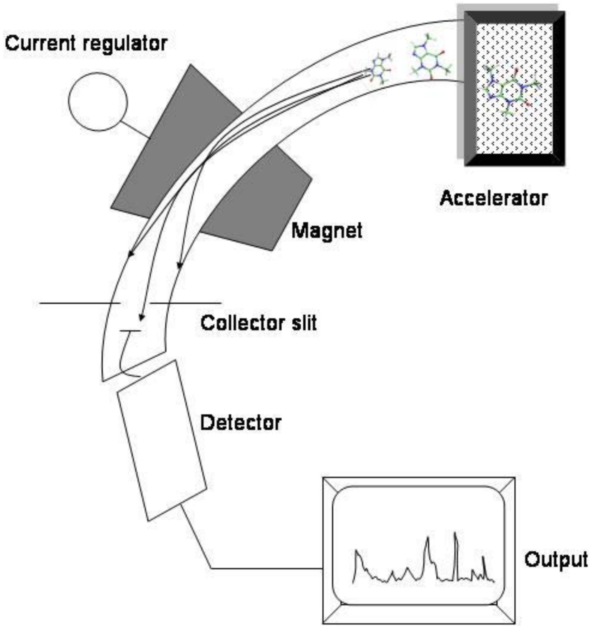


Figure 3.10 Schematic diagram of the mass spectrometer.

The GC-MS data however, can sometimes yield incomplete or false interpretations as given below (Burfield 2005).

1. The best GC detectors are still inferior to the discriminatory ability and sensitivity of the human nose (customer inference of quality). Many eluting (undesirable) compounds from GC columns can be distinguished (and sometimes identified by a trained nose) when the detector does not pick up any signal whatsoever. This is especially noticeable at present where prevailing adverse weather conditions, over-logging etc., in SE Asia, China, Indonesia, etc. have lead to the water-logging of some areas causing aromatic raw materials to rot: when distilled, the corresponding oils have a disagreeable smell but the standard GC-MS runs look fine.

2. The electronic detector does not respond uniformly to presented materials, and for accurate determinations, has to be calibrated against a 'pure' standard for each detected component. This involves a lot of work. 'Pure' standards (where available), as purchased from chemical companies standards, are often not that pure. For example, the purest analytical specification grade citronella (major component of *Eucalyptus citriodora* etc.) may contain polymeric material and have to be re-purified in the laboratory before use. Few aroma trade analysts carry out this accurate and exacting work.

For the present research, the level of accuracy presented by Varian Model GC-MS (described in chapter 5) was considered to be totally sufficient to conduct a series of comparative studies. The Varian GC-MS instrument comes with an built-in mass spectral library (NIST 2005). This is a powerful tool for reference and confirmation of all the familiar peaks obtained in regular GC-MS analysis. Since the current research is aimed at comparisons of peaks and not on investigating and thoroughly identifying all the peaks, it was found instructive to run the NIST program and identify some of the larger peaks for academic interest as shown in Figure 6.20 and 6.24.

4. DESIGN OF MICROWAVE ROTARY EXTRACTOR

This chapter briefly introduces the design features of the microwave extractor developed for the current research.

4.1 Microwave extraction in postharvest sequence of value addition

The postharvest losses in essential oil crops can be minimized by dove tailing farm operations with extraction, thereby significantly bypassing storage and drying steps of post-harvest processing. One method to achieve this in essential oil processing, involves loading the harvest in a ball-mill and effecting maceration with simultaneous microwave irradiation for solvent less volatile extraction and preparing the material for higher temperature non-volatile extraction procedures using green solvents.

The first conceptual design towards this end (Figure 4.1), involved a ball pestle macerator rotating inside microwave cavity (Ramanadhan 2003).

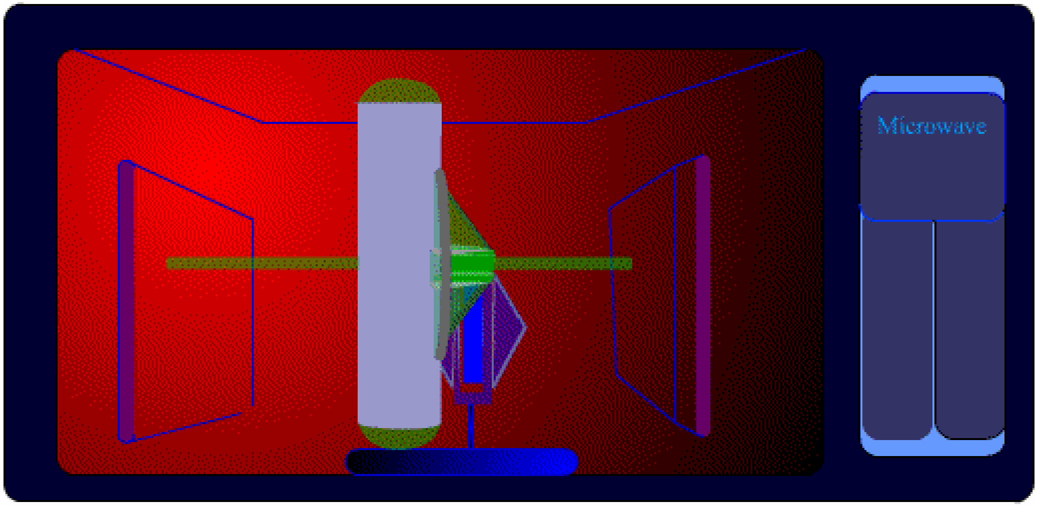


Figure 4.1 Initial design*of a ball pestle macerator that can dry, grind and extract simultaneously.
*(August 2003)

It was however found instructive to simplify the design to study only the microwave steps in isolation. The simplified design does not include maceration and hence starts with powdered raw material. The steps are shown schematically in the next section. But first, the criteria for the design are introduced below.

4.2 Design Prerequisites

In order to maintain spatial distribution of spice powders evenly within the microwave sample container (so as to maximize the microwave exposure of the bulk), there are two parameters (Table 4.1) that are to be ensured for the sample, namely, mixing of particulate matter and penetration of microwaves.

Table 4.1 The prerequisites for an efficient and scalable microwave extractor.

1) Segregation free distribution of particles	To enhance (for each particle), the probability of particulate proximity to the wall of sample holder where the microwave exposure is highest.
2) Thin layer spreading of the particles	To permit complete penetration of microwaves even at large water content.

Microwaves have a low penetration depth of about 1.5 cm in water at 2.45 GHz in ambient condition. To avoid thermal gradients originating from non-uniform absorption of microwaves (Figure 4.2), the current design begins with considering distribution of the spice powder randomly as a thin layer of about 1 cm thick (Figure

4.2) for sufficient duration of microwave exposure (by slow axial rotation) and simultaneously ensuring thorough mixing (Figure 4.3).

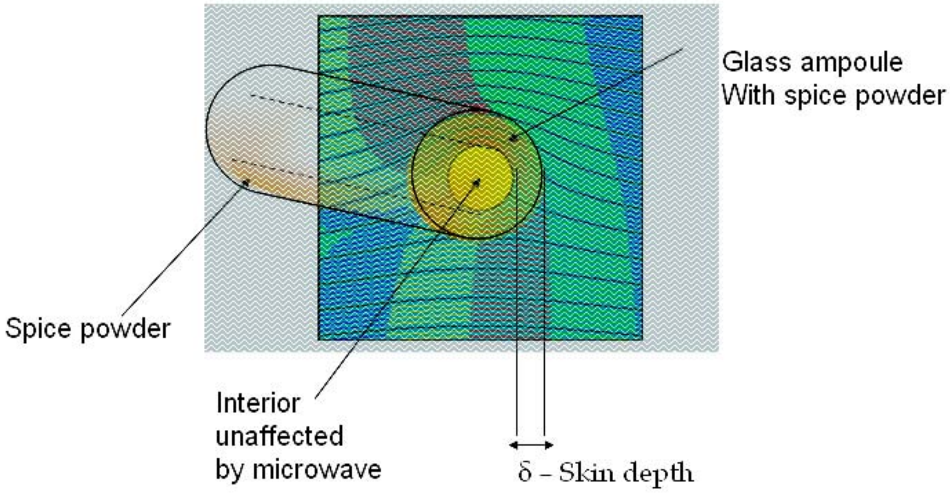


Figure. 4.2 The thin layer period during rotation is when microwave penetrate the most.

Mixing ensures every particle gets uniform opportunity to reach the surface of the ampoule (through a dynamic angle of repose). Maximum microwave energy is felt at the wall of the ampoule since the microwaves tend to decay from the point it interacts with an absorbing material. This is achieved by loading the spice powder in a flat bottomed ampoule and subjecting it to axial rotation (or tumbling). Segregation studies have shown for inclining cylinders, a flat barrier at the bottom leads to coarse and fine particle intermixing (Makse 1998) as seen in the Figure 4.3.

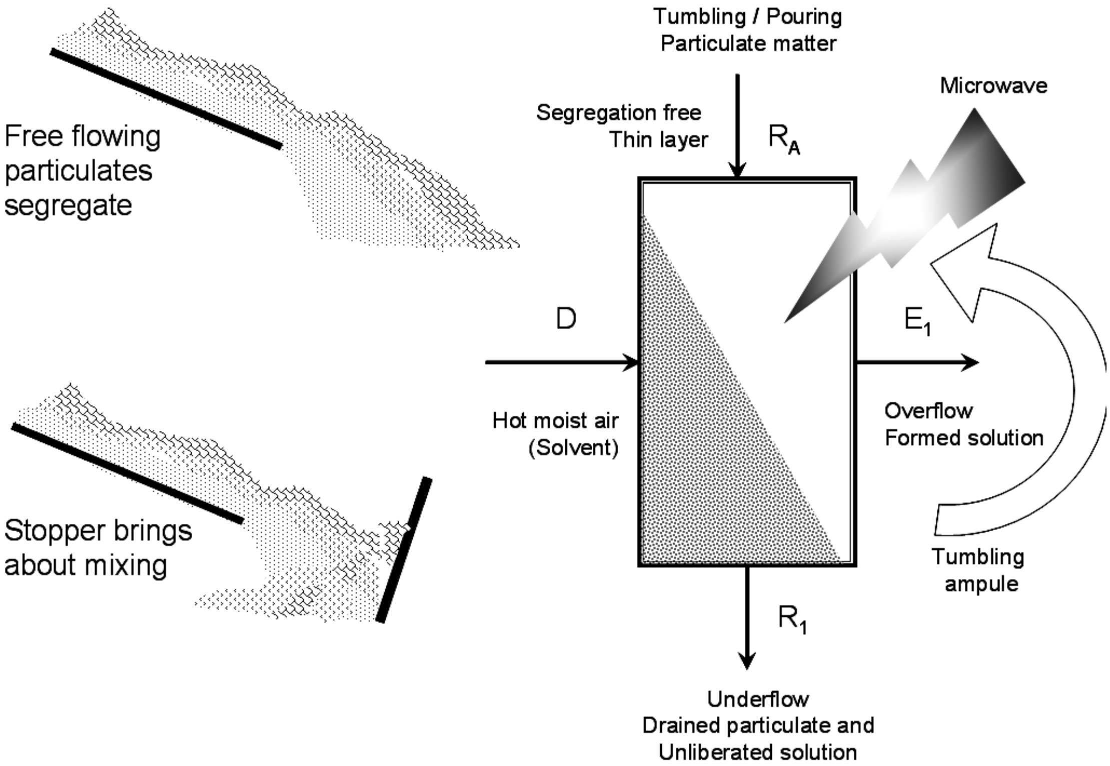


Figure 4.3 Diagram representing the microwave extractor.

Rotational motion in a cylindrical container is ideal for achieving conditions 1 and 2 used in Table 4.1. The requirements of thin layer and segregation free mixing are easily met as a cylindrical sample container rotates on its ends. When the wall of the container remains inclined for sufficient duration, Inclined plane thus created, first leads to trajectory separation of particles until they hit the bottom wall. At this stage the segregation is countered by a stopper effect leading to thorough mixing. When the motion is slow, the inclination also gives rise to thin layer formation. Initial experiments were performed to observe rotation-induced evolution of a simplified distribution of particulates by high resolution video recording using MotionMeter® digital imaging camera (Redlake MASD Inc. San Diego, CA, USA)). The photographs are as shown in Figure 4.4.

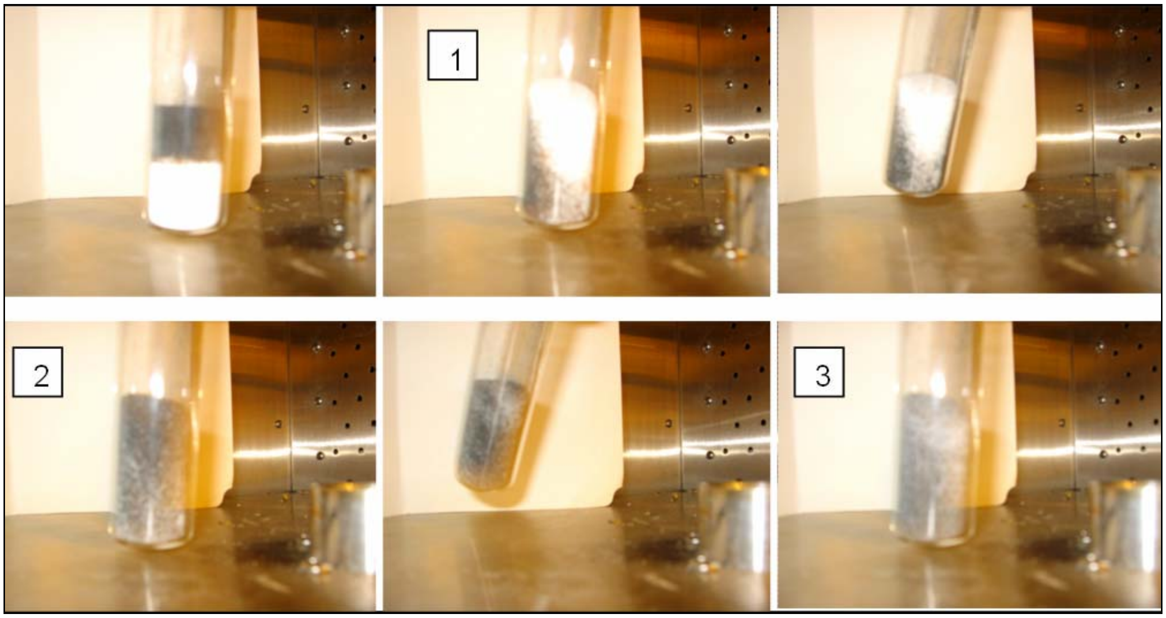


Figure 4.4 Photographs showing two color mixing: during rotation.

4.3 Design Criteria for Rotary Microwave Extractor

Following are the criteria for designing the rotary extractor for particulate exposure to microwaves:

1. Scalable design for ultimate combination of postharvest unit operations viz. grinding drying and extraction of natural products in a single step;
2. The uniform distribution of plant material exposed to microwave;
3. Thin layer exposure of plant matter dynamically replenished so as to afford equal opportunity to every piece of plant matter for microwave exposure; and
4. To have the spice loaded ampoule paired symmetrically (Figure 4.5) with a reference ampoule containing water so as to estimate the microwave field strength felt at the region of extraction from temperature rise measurement of water whose physical properties are well documented.

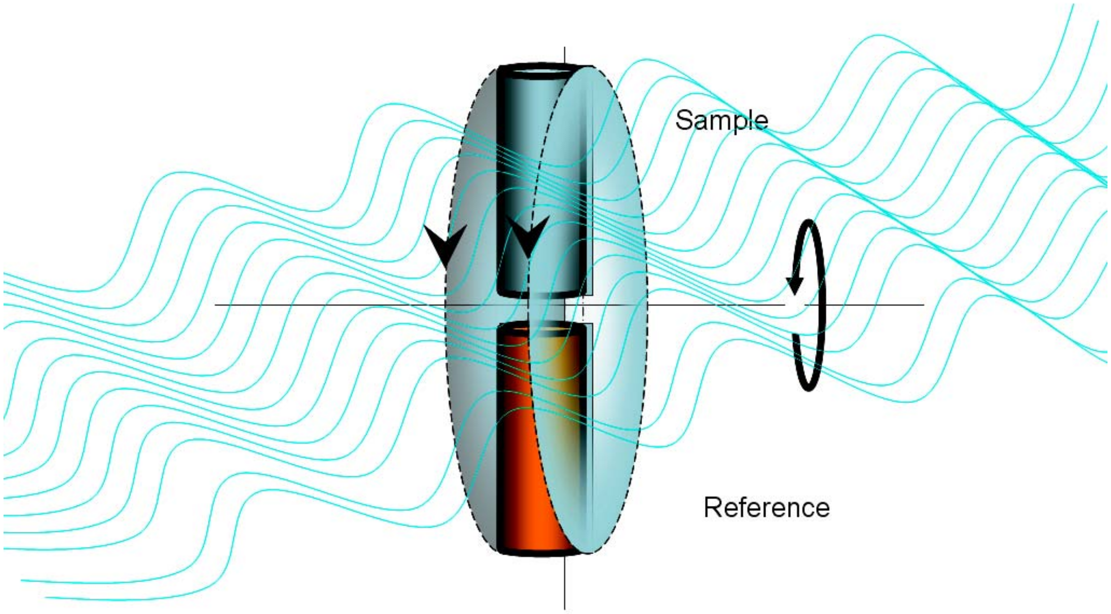


Figure 4.5. Symmetrical arrangement of the ampoules containing the sample and the reference permits the study of field and other microwave properties exactly at the region of the processing.

4.4 Design for the Current Research

The microwave rotational extractor designed and developed in the current research had a central sliding holder for two glass ampoules (sample and reference containers) as shown in Figure 4.6. The dimensions of the ampoule were: length 11.09 cm; radius 1.33 cm; and, height 0.36 cm.

In the current design (Figures 4.3 and 4.6), heated air serves as the solvent. As the particulates tumble, they get washed by the heated stream of air whose over-flow concentration gradually increases in terms of mass fraction of essential oils. The underflow consists of particulates partially drained of essential oils and a finite quantity of trapped (un-liberated) hot air containing essential oil vapors. The underflow falls to the bottom of the ampoule and begins the next cycle of thin layer exposure to microwaves. The rotating ampoule at low rpm (5 rpm) gives long periods of thin layer formation as the spice powder slides down the ampoule side. The speed is controlled using a voltage regulator and the desirable speed of 5 rpm, is found to correspond to 6V. This feature gives the rotary extractor an ability to achieve a good thin layer formation and thorough mixing.

Figure 4.6 Photograph of microwave rotational extractor.

4.5 Design Benefits in the Current Configuration

The current design has the following benefits over any other reported essential oil extraction model:

1. uniform volumetric heating of spice material;
2. complete mixing of the substrate;

3. optimum opportunity for every particle to absorb microwave;
4. thin layer exposure providing total penetration of microwaves;
5. portable and very low cost design;
6. rapid volatile sample withdrawal (few seconds);
7. GC-MS analysis easy to compare with other extraction procedures; and
8. symmetry of the rotary extractor (Figure 4.7) permits accurate evaluation of microwave properties of the substrate such as penetration depth power absorbed and permittivity (Figure 4.6).

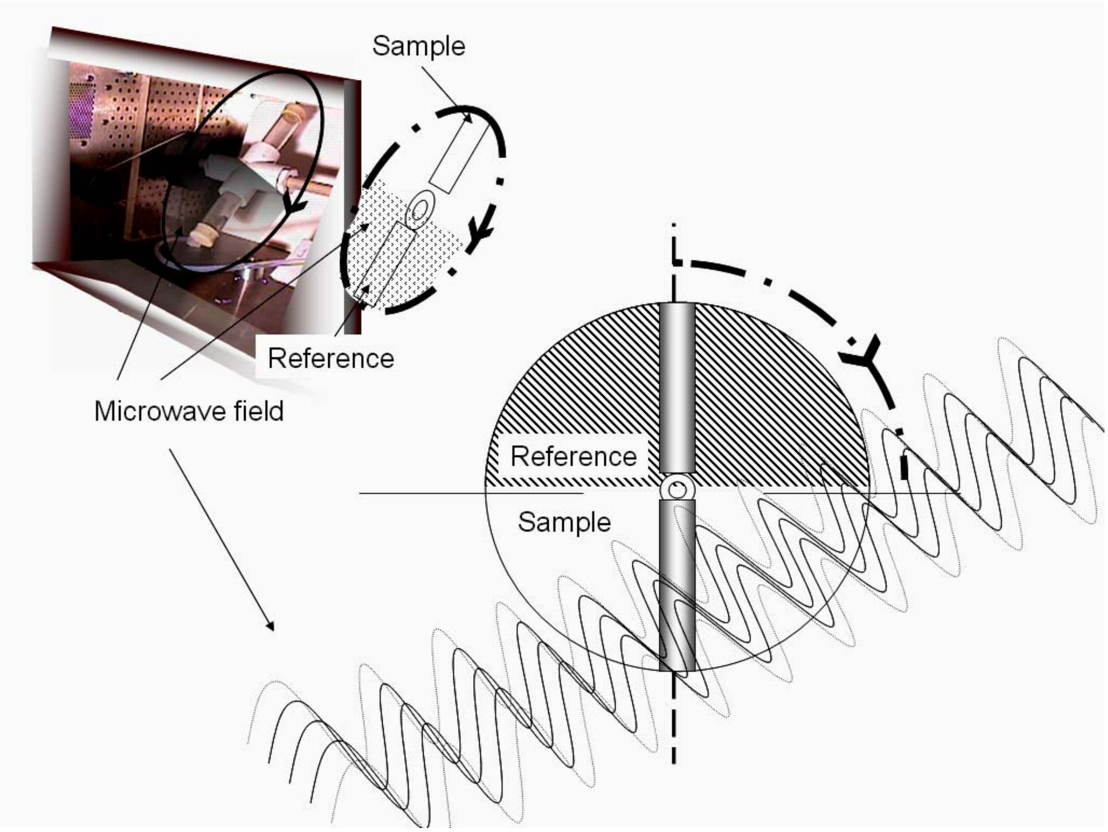


Figure 4.7 Ampoules cut the microwave field symmetrically inside the MW oven.

The reference and the sample cut exactly the same region during their rotation. This is the salient feature of the design and has potential for extensive applications.

5. MATERIALS AND METHODS

The materials and equipments used in the current research are described below, followed by the methodology of the experiment. A diagrammatic representation of the experiment is given in Figure 5.16.

5.1 Materials and Equipment

The materials and equipments used in the current research are described in the following sections. All the instruments and equipments used in the current research were housed within the college of engineering, University of Saskatchewan.

5.1.1 Spice powder

Spices used in the current research were black pepper powder and coriander powder (particle size of 350 µm cf. Appendix B). Powder as purchased from retail outlets were more desirable for current experiment since its volatile components are likely to have attained an equilibrium during its residence in shelf. The freshly powdered materials rapidly lose volatiles and may lead to high error in comparative studies on essential oil extraction. Black pepper corn and coriander seeds were used in the load to improve flowability. The spices for the extraction studies were obtained from local retail outlets in the form of dried powder. Both were from Suraj brand, processed for Westfair Foods Ltd, Calgary AB. The batch numbers for the powders were: black pepper: lot 308F-6454 and coriander: lot 303F-6464.

5.1.2 Particle size analyses

The particle size analysis was carried out in Mastersizer S- Longbed Version 2.19. (Malvern Instruments Ltd., Worcestershire, UK) (Figure 5.1). Malvern Mastersizer S-Longbed version 2.19 is a particle size analyzer that measures particle diameters and plots the range in terms of percentage distribution Appendix B.

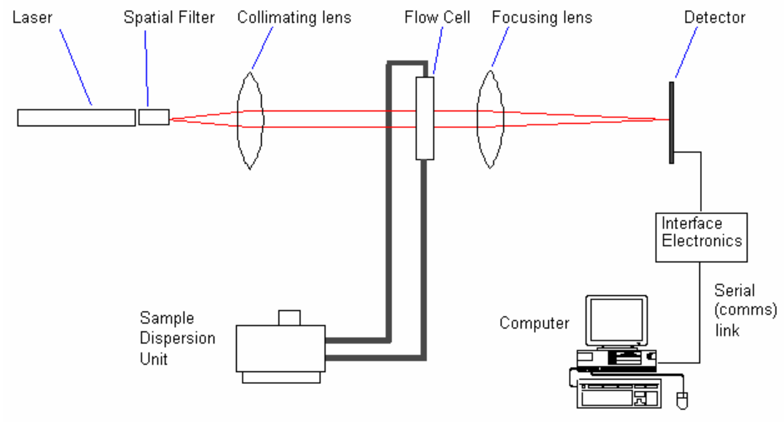


Figure.5.1 Malvern Mastersizer S Longbed Version 2.19.

5.1.3 Rotary set-up.

The rotational extraction module (Figure 5.2) serves three major purposes in the experiment. It enables uniform particulate distribution and presents a thin layer for microwave exposure.

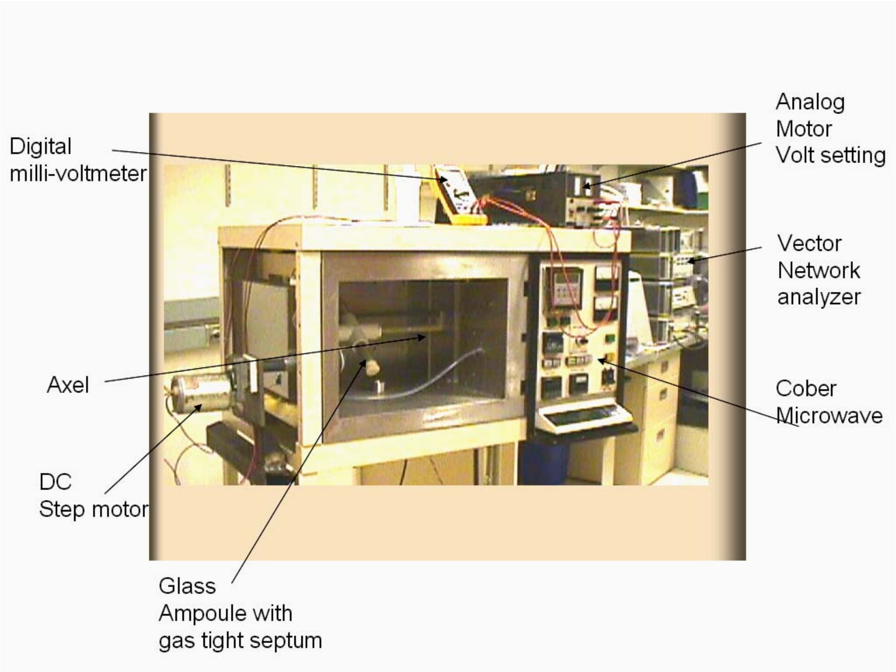


Figure 5.2 The modified microwave with rotational extractor (in the background: the experimental area).

The rotational extraction also helps in the variation of field owing to its axel serving as a slider for the ampoule holders.

The rotational module developed for the experiment consisted of a PVC rod (214.57 mm long) serving as an axel upon which was fitted, a sliding sample holder with a capacity to hold two glass ampoules (110.91mm height: 28.56 mm diameter) transversely on opposite sides of the axel (Figure 5.2).

5.1.4 High speed camera for particle flow observation.

The particle distribution in the ampoule and rotational speed studies were made using a MotionMeter® high speed digital imaging camera (Redlake MASD Inc. San Diego, CA, USA). The MotionMeter® is a handheld, digital, slow motion camera for capturing high speed events. The device is ideal for visually diagnosing particulate events in a glass rotary setup.

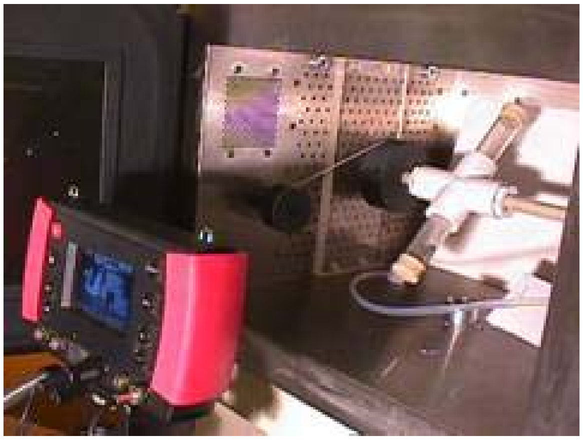


Figure 5.3 Motionmeter® enables frame by frame analysis of rapid events.

The (Motionmeter® was used (Figures 5.3 and 5.4) to record the rotational motion of particulate matter and arrive at the speed when thin layer formation is highest as well as uniform.

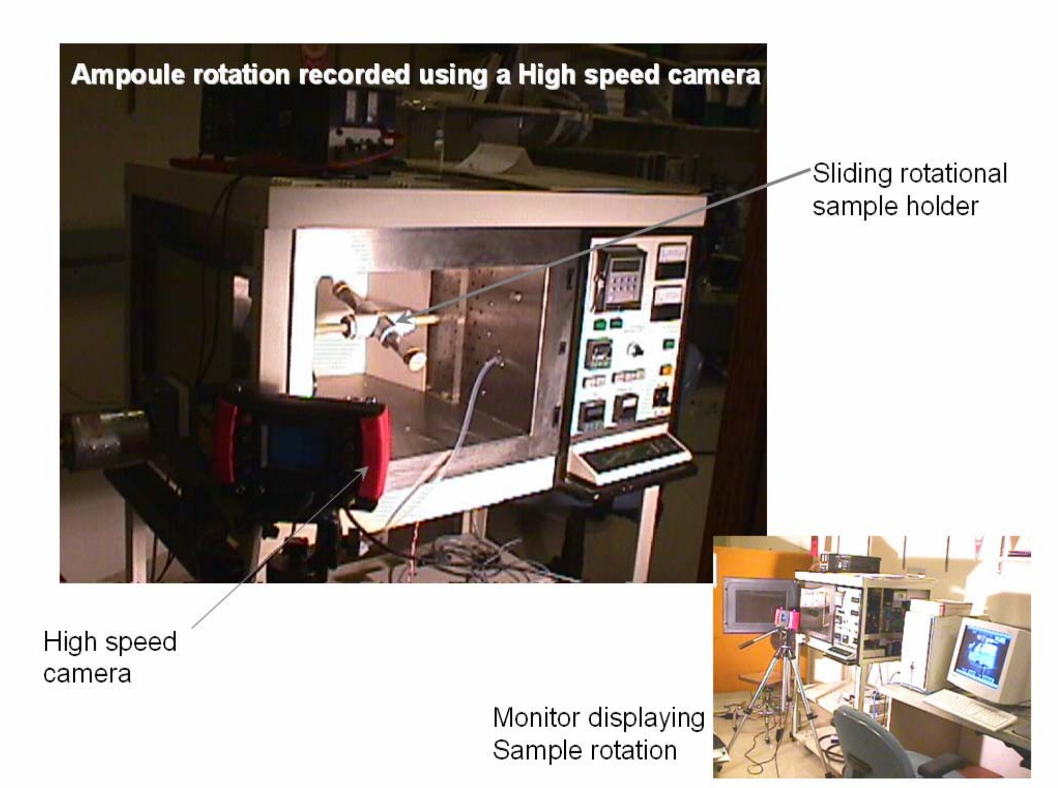


Figure 5.4 The oven door was removed and rotation was closely studied.

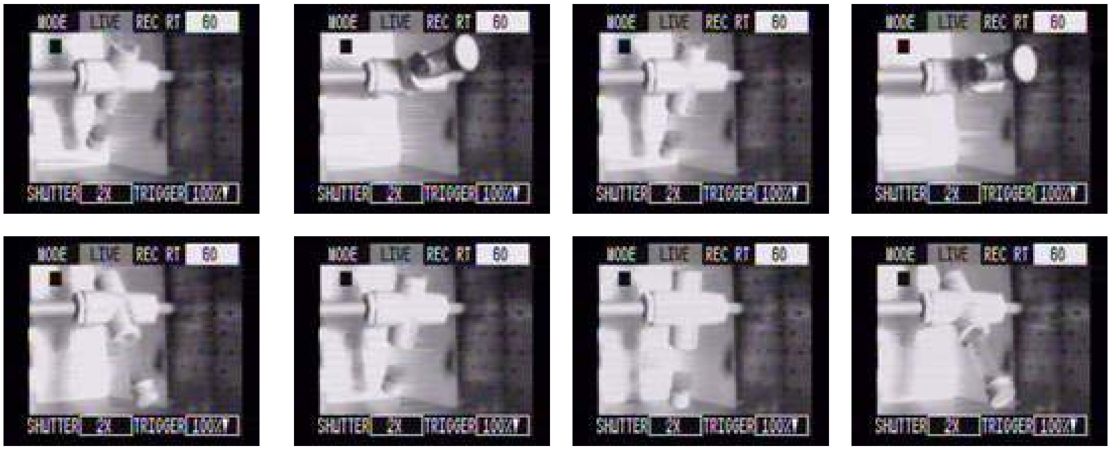


Figure 5.5 The video recording of eight rotational speeds was analyzed to study the flowability and thin layer formation quantitatively.

The Figure 5.5 shows the video clips of eight rotational speeds taken using the Motionmeter® as detailed in chapter 6. On observing the characteristics of the particles during rotation, it was decided to hold the speed of rotation meeting all the stated criteria for the rotational module, as constant throughout the experiment. Ampoule rotational speed of 5 rpm was chosen as the ideal speed for uniform and largest thin layer distribution.

5.1.5 Step motor

An external DC step motor Model 141-210 Zenith Co. Sanford, NC and pulley arrangement was made for axial rotation of the extractor, (Figure. 5.2). The DC step motor had uniform speed ranging from 5 rpm to 15 rpm. The motor conveyed its torque through a wheel-pulley arrangement that could be modified for gaining higher rotational speeds. An analog voltage adjustment and digital millivoltmeter display was used to set the speed of the motor.

5.1.6 Microwave frequency measurement

The COBER™ microwave LBM 1.2 A/ 7296 (Cober Electronics, Inc.,Norwalk, CT, USA) used in the current research was manufactured around 1987 (the earliest manufacturer of MW ovens). There were no records of the magnetron specification available and hence, the frequency of the oven was to be established.

The very early microwave ovens were built with magnetrons of frequency 915 MHz (whereas recent models operate at 2.45 GHz). Thus, it was imperative to perform the frequency measurement using a signal analyzer. The measurement also gave a fair idea of the range of frequencies that the magnetron spans so as to know the operating frequency for calculations.

Magnetrons can range about 200 MHz above and below the stated levels. HP Spectrum analyzer 70000A; 70206A system graphics display (Agilent Technologies, Inc., Palo Alto, CA, USA) was used for frequency measurements. Initial measurement was made using a flexible RG coaxial cable on fugitive signals from a gap made by marginally loose oven door (Figure 5.6 inset).

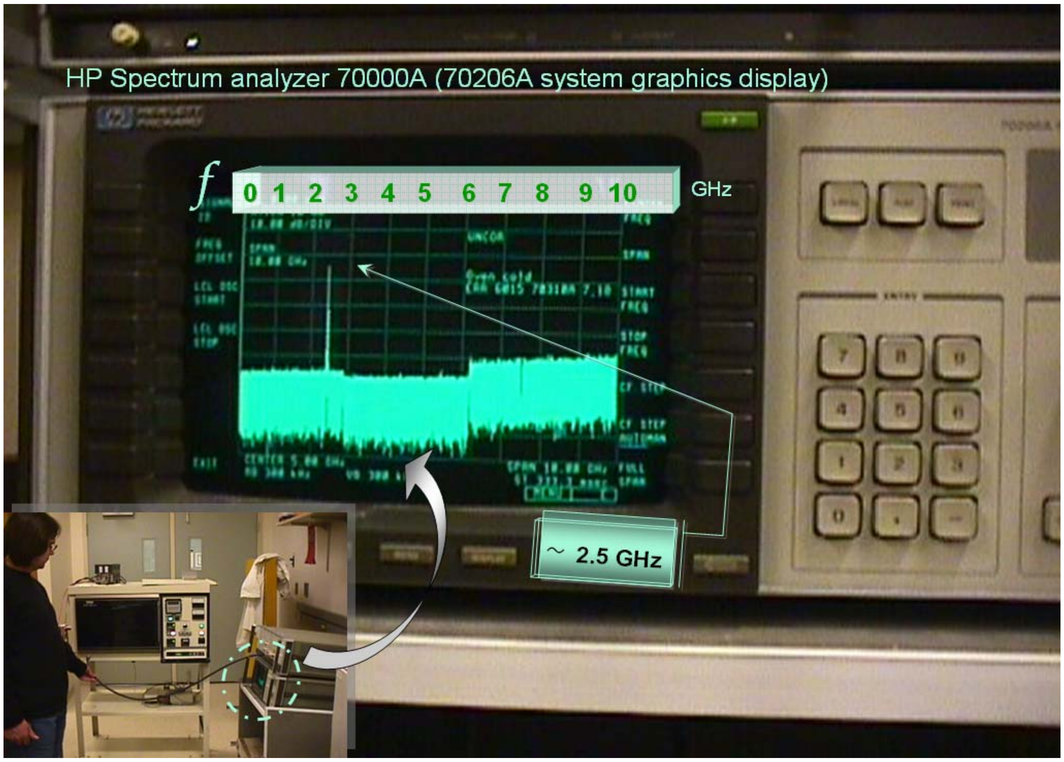


Figure 5.6 The measurement of frequency using HP signal analyzer HP70000A.

The measurement confirmed the frequency of the COBER™ microwave oven to be about 2.5 GHz and not 915 MHz expected of an older microwave oven. The frequency was further measured using a more sensitive antenna for obtaining higher accuracy of the frequency value.

The finer measurement was made using a loop probe to get a sensitivity of 100 Hz. The coaxial cable (2 ft 0.141 semirigid Beldon, NY, USA) was connected to the spectrum analyzer and the sensing end was a loop probe 1 inch diameter. The method and its accuracy is described in section 6.1.1.

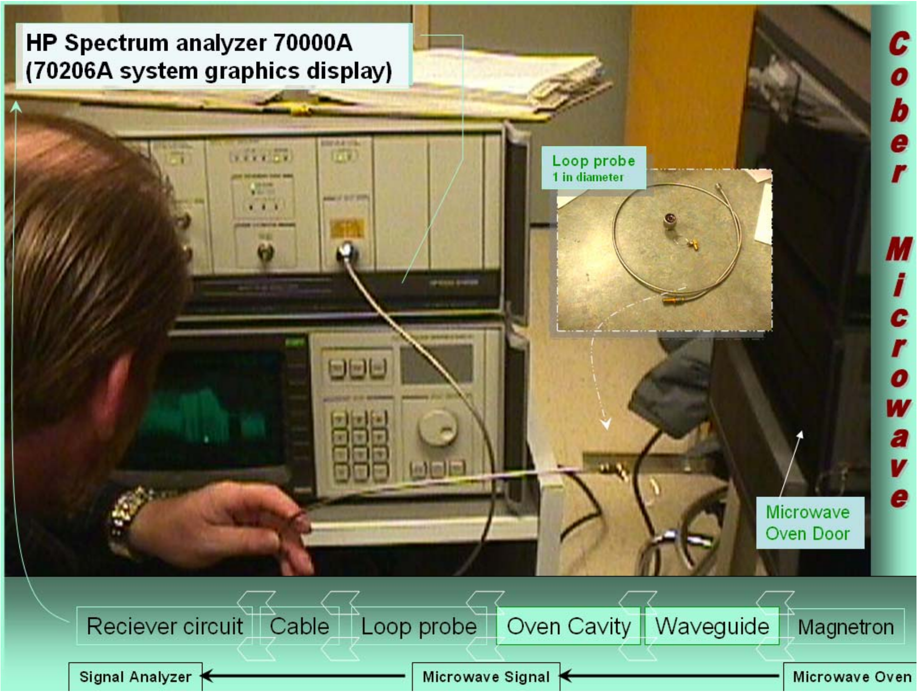


Figure 5.7 HP signal analyzer measuring the microwave frequency of COBER™ oven.

Figure 5.8 Signal peaking at 2.46 GHz (oven frequency) just above 60 dBm (~1kW).

5.1.7 Vector network analyzer.

HP network analyzer 8510B (Agilent Technologies, Inc., Palo Alto, CA, USA) was used for Dielectric measurements (Figure. 5.9). This test instrument is used to measure the scattering parameters (magnitude and phase of one- or two-port microwave networks from 0.05 to 26.5 GHz). From scattering parameter, derived quantities such as impedance, return loss and permittivity are obtained. The values obtained would indicate the extent to which material heats and to what depth the field can penetrate.

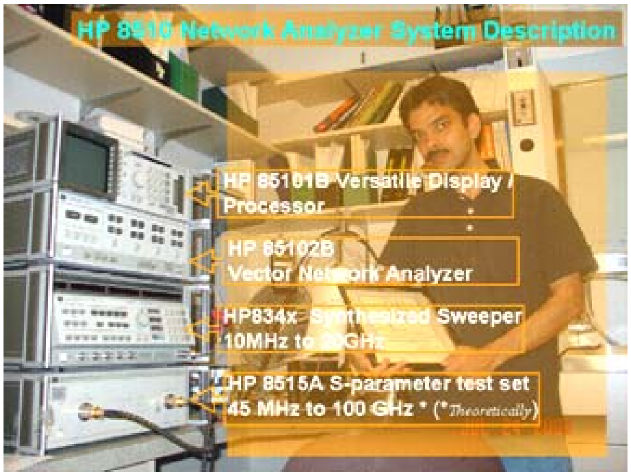


Figure 5.9 HP Vector network analyzer used for measuring permittivity.

5.1.8 The microwave oven

The microwave source was from COBER™ Laboratory Microwave (Cober Electronics, Inc.,Norwalk, CT, USA) model LBM 1.2 A/ 7296 (Microwave/convection) 1988, Power (0 to 1200 W) shown in Figure. 5.2 and 5.4. This oven has power controls that can be used to vary the power analogously. It was modified to accommodate the rotary extractor set-up.

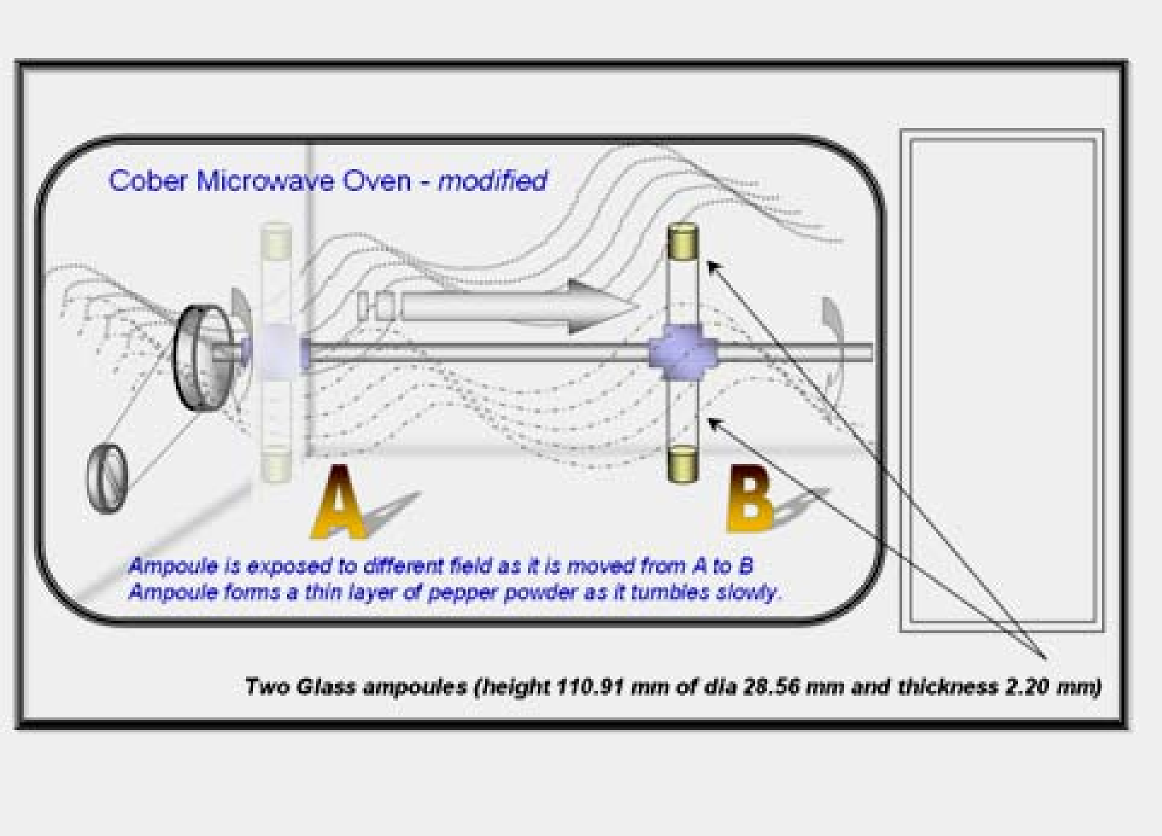
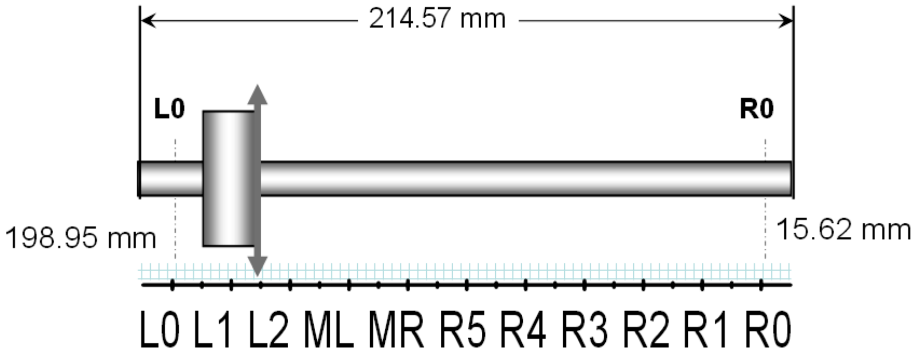


Figure 5.10 The sliding extractor positioned accurately to receive a specific microwave field strength.

Multimode microwave ovens inherently lead to field variations within the oven cavity. By moving the sample from one position to another inside the oven is tantamount to varying the microwave field to which sample is exposed (Figure 5.10). The axle was divided into eleven regions, corresponding to eleven different fields. The 'Location' and the field levels are shown below (Figure 5.11) The schematics of the sliding ampoule holder gives the 'Positions', respective microwave field strengths and the location of the microwave field along the axle in mm measured from right extreme of the oven cavity.



R0 [**81.84 V/m**] *is located* 15.62 mm *from the cavity wall at right extreme*

R1[**83.19 V/m**] is at 25.62 mm
R2[**87.41 V/m**] 35.62 mm
R3[**86.83 V/m**] 50.62 mm
R4[**93.46 V/m**] 65.62 mm
R5[**92.14 V/m**] 80.62 mm

MR[**85.32 V/m**] 95.62 mm
ML[**84.56 V/m**] 171.78 mm
L2 [**81.83 V/m**] 180.80 mm
L1 [**81.10 V/m**] 189.80 mm
L0 [**58.64 V/m**] 198.95 mm

POSITION SYMBOL [**MICROWAVE FIELD V/m**] LOCATION (**mm**) FROM RIGHT

Figure 5.11 The experimental positions taken by the sliding extractor and the respective microwave field strengths in V/m.

5.1.9 Infrared pyrometer/ thermometer

Infrared thermometer is used for remote temperature measurements. It is also referred to as pyrometer since it finds a unique application in fire studies. The thermometer used in the current research was a wide range infrared thermometer (Extech Instruments Co. Waltham, MA, USA). The method of its use is shown in the Figure 5.12. Focusing of the thermometer on a hot spot leads to a digital temperature output on the handheld device.

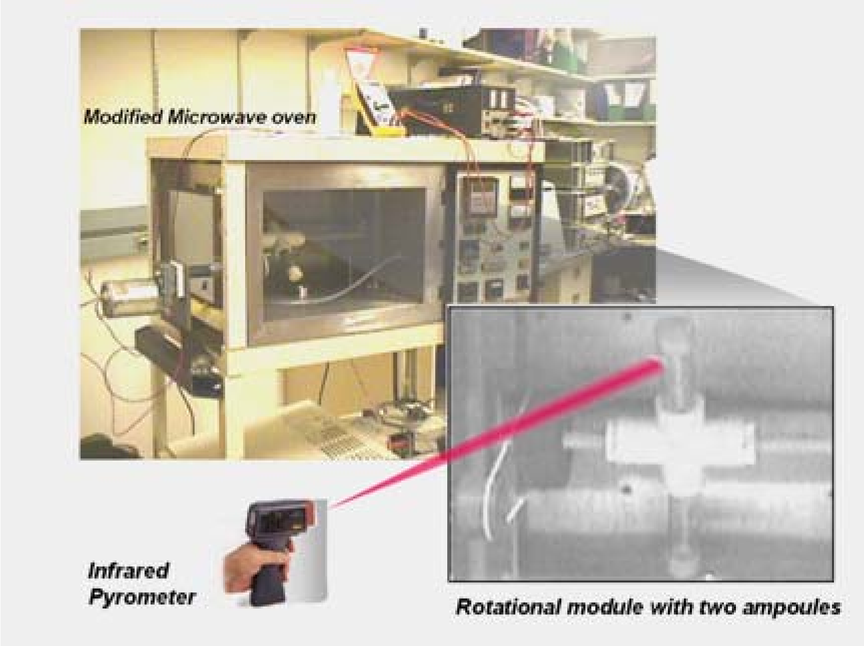


Figure 5.12 The rotational setup and IR pyrometer for temperature measurement.

5.1.10 Solid phase micro extractor (SPME)

The microwave extracted volatile was withdrawn using SPME with carboxen polymer (Figure 5.13).

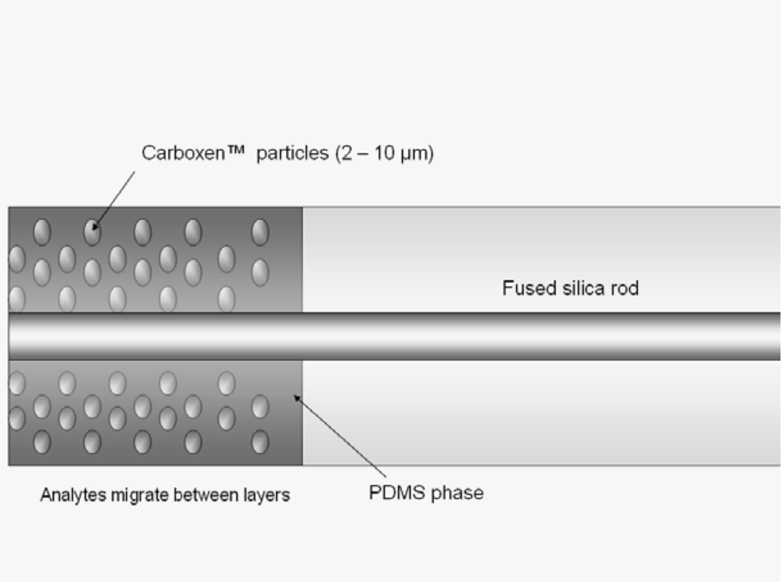


Figure 5.13 Diagram of the Carboxen Fiber of the SPME.

The carboxen fibre has unique advantages for the molecules studied in the current research that have a mass about 200 amu. The SPME needle has carboxen polymer coating on it which can adsorb volatiles and retain them until desorbed inside a gas chromatographic equipment. The gas tight rubber septum of the ampoule enables smooth withdrawal of volatiles. The polymer coated needle adsorbs volatiles and retains it until it is released inside the gas chromatograph. The polymer fibre chosen for the current work was carboxen upon PDMS.

5.1.11 Gas chromatography-mass spectrometry. The essential oil profile was obtained using a Varian Saturn GC-MS (Varian, Inc. Palo Alto, CA, USA).

Figure 5.14 Varian gas chromatograph- mass spectrometer.

Gas chromatograph and mass spectrometer which are two stand alone analytical instruments that can be seamlessly connected to perform separation and identification of chemical compounds. The chromatograph instrument comes with an injection port for SPME desorption.

5.2 Procedure for Microwave Extraction – Development and Standardization

This involved measurements that were made to arrive at the conditions of the experiment to be held/considered constant throughout the research. The precision, accuracy and resolution of all the measurements made in the current research are also described in this section.

5.2.1 Experimental sequence

The glass ampoule was loaded with spice powder and sealed with a gas tight rubber septum. The load of the powder and ratio of corn to powder was based on flowability of the powder determined using the Motionmeter© video recordings. The ampoules were fitted into the holder so as to have one reference and a sample on each side. The rotary extractor was set at a rotational speed of 5 rpm at which it gave rise to a thin layer of powder for the longest period. The microwave power was set to a low value of 3.9 COBER™ proprietary units. The magnetron was powered on immediately as could be verified using a spectrum analyzer and hence the switching on time is taken as time zero seconds. Once the extraction was carried out, the ampoule temperature was measured using IR thermometer. It was then detached from the rotational extractor and transported to the GC-MS lab for analysis.

5.2.2 Parameters varied in black pepper extraction

The parameters varied towards the extraction studies are described in this section.
The main parameter categories were as follows:

1. microwave field;
2. repeated extraction and analysis on a single sample;
3. power levels; and
4. substrates

5.2.2.1 Microwave field impact

The ampoule holder was positioned across the axle at eleven different points. Each point exposes the tumbling ampoule to different microwave field strength. The field strength was calculated by measuring the heating of distilled water at each position. By comparing the GC-MS data of each extract, a pattern can be arrived at for the impact of different microwave fields on extraction.

5.2.2.2 Exposure time and substrate variation

Forty experiments were done at different microwave exposure times to study the effect of sampling and analysis on the same sample. Five experiments were performed by changing the substrates content (such as inclusion of alcohol). Four experiments were performed to see the effect of high added water levels upto three times the mass of sample.

5.2.2.3 Power levels

Two power levels were used: For all the triplicate experiments at eleven different fields, the power level was kept at moderate (3.9 units). Eighteen experiments were done at the maximum power of the oven (9 units) with inclusion of 15% water content.

5.2.3 Parameters varied in coriander extraction

The extraction of coriander was carried out in triplicates for five different fields (81.10 V/m, 84.56V/m, 92.14 V/m, 86.83 V/m, and 87.41 V/m). The power was maintained constant at 3.9 as in the case of pepper triplicate experiments. The time was set as follows: 2 seconds for rotation of the ampoule from lateral position, 3 seconds for oven main power and 4 minutes for the microwave oven. The SPME conditions were 4 minutes at 4 cm for adsorption and 2 minutes at 2 cm at the injector port for desorption.

5.3 Microwave Field Measurement

At the end of specific time of microwave operation, the oven was switched off and the temperature of both the ampoules was measured using infrared camera. The reference (water) temperature rise ΔT_w was used for arriving at the electric field coupled by the two ampoules.

The diagrammatic representation of the field measurement is given in Figure 5.15. The calculation of electric field involved the measurement of temperature rise using an IR pyrometer, the duration of extraction, dielectric loss in a vector network analyzer and the frequency of the oven using a signal analyzer.

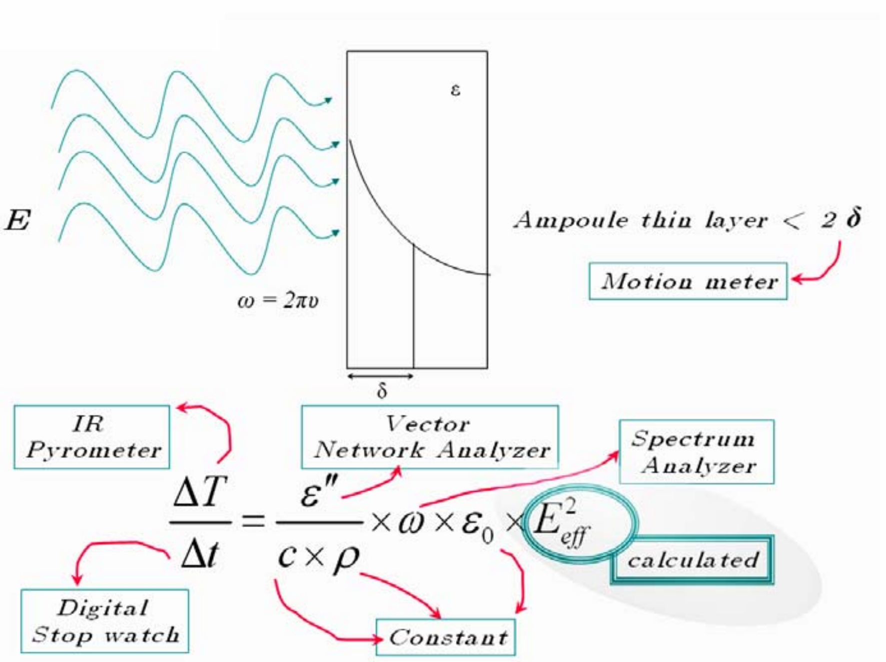


Figure 5.15 The calculation of electric field – schematic description.

The calculations of microwave field from temperature rise measurements were based on the following equations. The starting point of the calculation was the thermochemical heat equation:

$$\Delta Q = C_p m \Delta T F \tag{5.1}$$

The microwave power equation is as follows:

$$\Delta P = \omega \varepsilon_0 \varepsilon'' V E^2 \tag{5.2}$$

The absorbed energy is given by

$$\Delta t.\Delta P = \omega \varepsilon_0 \varepsilon'' V E^2 \Delta t \quad (5.3)$$

Q= Heat generated, J

C_p= Specific heat of water, $kJkg^{-1}K$

m= mass of water taken, Kg .

T= the measured temperature, K

F= empirical factor that accounts of heat from other sources.

P= Power absorbed, W

E= Electric field coupled by the ampoules, Vm^{-1}

ε_0 = the absolute electric permittivity of free space, 8.86 x 10^{-6} Fm^{-1}

ε'' = Dielectric loss factor

$\omega = 2\pi\upsilon$, where υ is the frequency of microwave oven, Hz.

The right hand side of equations 5.3 and 5.1 are equal. By equating the two right-hand sides (5.3 and 5.1) and using the literature values for the constants, the microwave field (V/m) can be estimated.

This is the field cut by the spice powder during the microwave exposure for Δt seconds and microwave power P.

5.4 The analysis of the extracted volatiles

The extracted volatiles from each run was held/stored in the gas tight ampoule and transported to the GC-MS lab where it was analyzed for its volatile profile using SPME for withdrawing the volatiles and using GC-MS for chemical analysis.

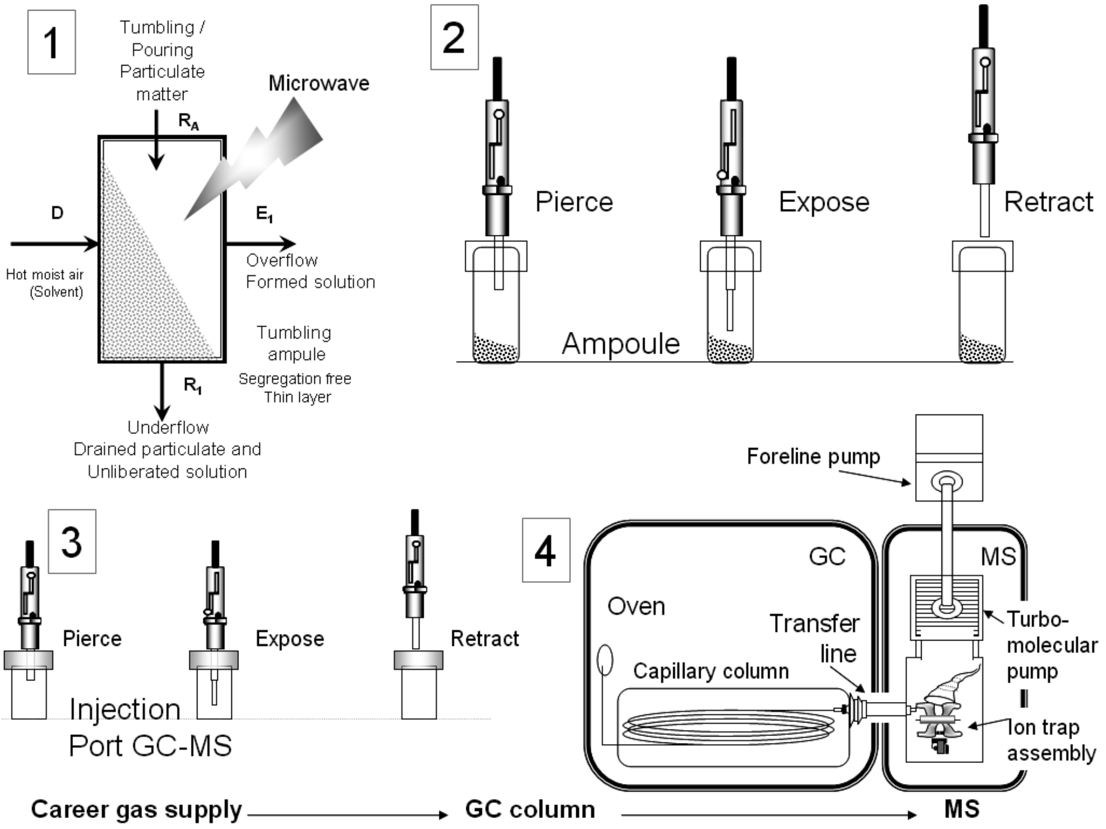


Figure 5.16 The extraction, sampling and analysis steps - starting with microwave extraction followed by SPME sampling culminating in GC-MS analysis.

The profile for each electric field E was plotted as a three dimensional plot with coordinates for retention time, extent of extraction and microwave field.

The plot of microwave field versus essential oil is valuable information, as it is an intrinsic information independent of extraction equipment but only the particle size. The data analysis steps that yield a unique value for each extraction in terms of ion counts are shown in Figure 5.17. Towards summarizing the experiments, Figures 1.1, 5.15, 5.16 and Figure 5.17 are the four diagrams, together describing the whole research process.

5.4 Data Analysis

The GC-MS profile essentially gives the retention times of various compounds with the respective peak intensities. In the experiment designed for comparative analysis of extraction, it was found useful to have a single unique quantity to rate the extraction.

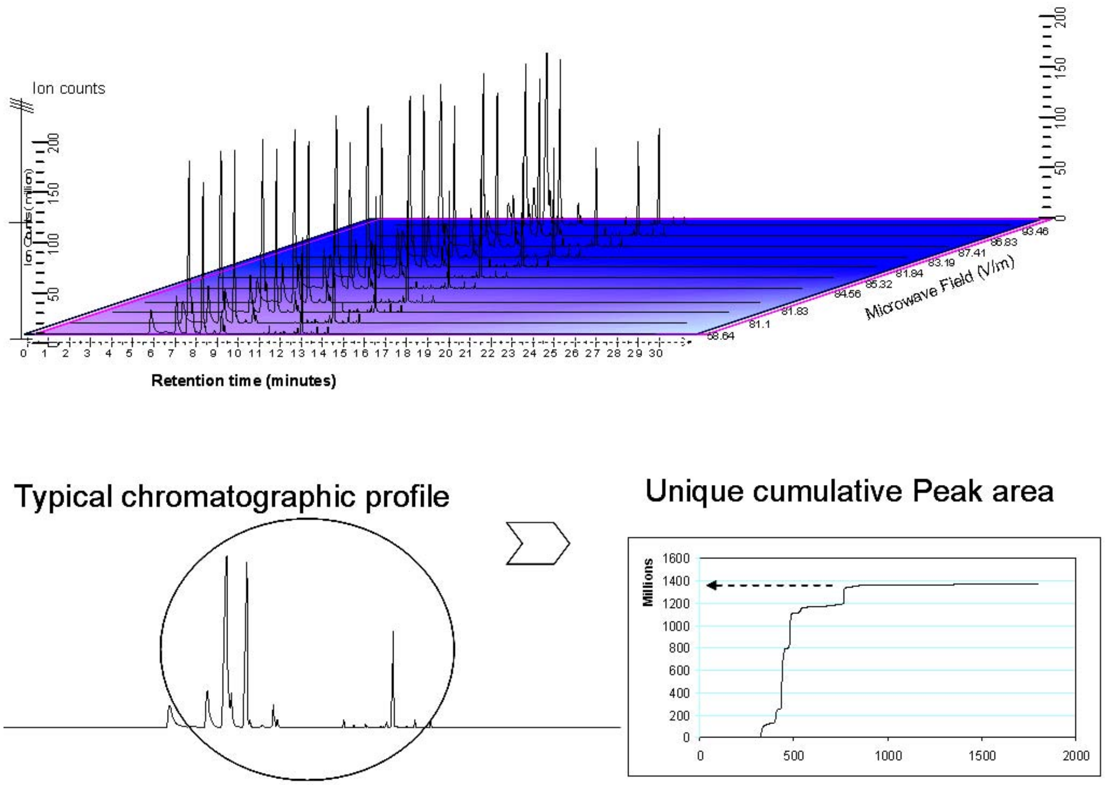


Figure 5.17 The Data analysis steps – starting with collecting the GC-MS and converting each chromatogram into a cumulative peak area plot that yield a unique value for each extraction.

Hence, the GC-MS profile was converted to a cumulative plot and the value of the cumulative at the end of GC-MS time scale was taken as the extraction measure in the units of ion counts.

6. EXPERIMENTAL RESULTS AND DISCUSSION

Unlike chemical unit operations, microwave processing and its measurements are immensely difficult to perform (owing to sensitive nature of microwaves and its susceptibility to high perturbation), a unique experimental design was developed to enable microwave measurements. The aim of the research was to test the feasibility of this procedure and gain insight into the effect of microwave field on essential oil extraction. In this chapter, results of the experiments are presented. At the outset, it is felt important to delve on the results leading to standardization of the procedure itself.

6.1 Standardization of Microwave Extraction Parameters

This involved measurements that were made to arrive at the conditions of the experiment to be considered constant. The precision, accuracy and resolution of all the measurements made in the current research are also described in this section.

Precision refers to repeatability; whereas accuracy refers to the closeness of the measurement to reality and as the representation of the absolute value. Resolution refers to the degree or decimal places to which the measurement can be reliably stated. The current research is a green field work. There is no reported literature on similar electric field measurements and effect of its variation on extraction of essential oils. As such, a novel microwave extraction method was conceived and a procedure for sampling and testing the extracted essential oil was developed ab initio. The research was designed so as to hold all the steps of the experiment, except the microwave field, under constant condition. The constant condition was arrived based on initial preliminary measurements. The results of extraction were compared using a GC-MS profile.

It is expected that scope for further improvization would exist in every step of the current experiment which was designed intuitively to be monolithic and integrated so as to address a complex subject in its entirety.

The experimental procedure was found to be precise as seen in the GC-MS profiles obtained (Figure 6.1). GC-MS profiles are shown in Figures 6.1 and 6.2 for essential oils from two independent experiments done under identical conditions.
A typical experiment consisted of twenty steps, namely:

1. sampling;
2. weighing first ampoule;
3. weighing sample;
4. weighing second ampoule;
5. weighing water reference;
6. positioning the ampoule with sample and reference in microwave oven;
7. measuring temperature of water reference using IR pyrometer;
8. starting motor and beginning timer;
9. two seconds after step 8 - Switching on the microwave control panel;
10. three seconds after step 6 - Switching on the microwave power;
11. at final time – stopping the microwave oven;
12. measuring temperature using IR pyrometer;
13. releasing the ampoule and shaking it 10 times;
14. transporting to the GC-MS lab;
15. performing solid phase micro extraction set the length;
16. performing solid phase micro extraction set the time;
17. entering sample identification details in the GC-MS and stabilizing;
18. performing SPME desorption set length;
19. performing SPME desorption set time; and
20. performing GC-MS;

The chromatograms in Figure 6.1 and 6.2 show the comparison between two individual experiments on the basis of the final step of performing GC-MS, which accumulates all the errors occurring in earlier (mostly) manual steps.

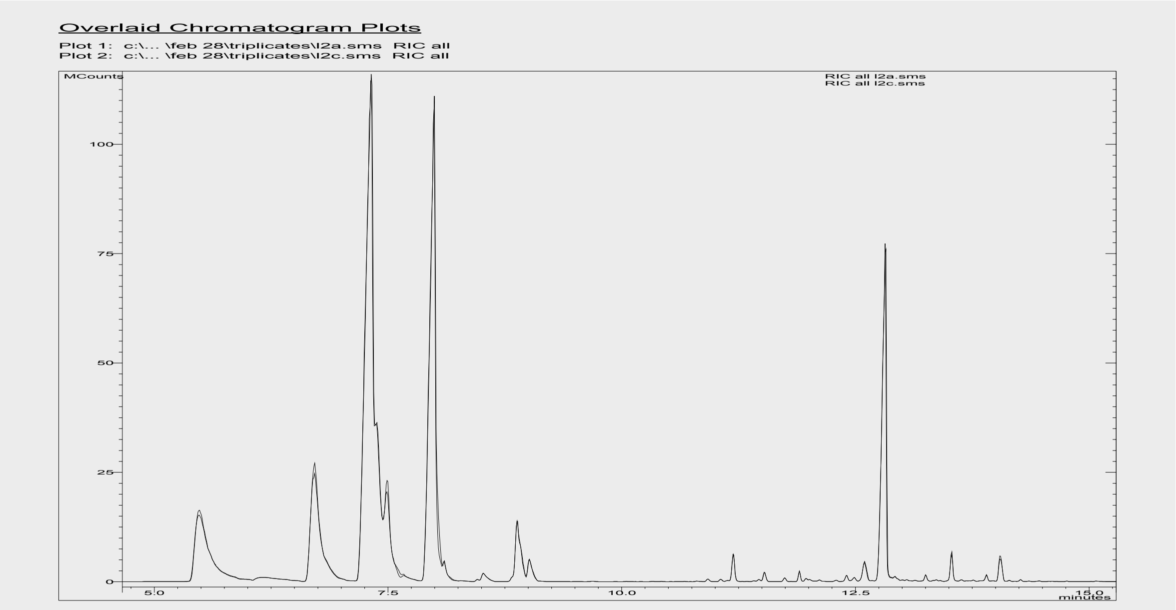


Figure 6.1 Two chromatograms from duplicate experiments overlaid.

Figure 6.1 shows two chromatograms laid one upon the other and Figure 6.2 has the same two chromatograms stacked. The high reproducibility of the results is seen in these chromatograms.

Although, the two profiles are examples of the best repeatability, even in the same triplicate experiment there was a marginal variation in one of the chromatograms (the shape of the peaks however remained identical). Further, within the variations among the peaks in the chromatograms, certain peaks remained concordant (peaks ‘b’ indicated by dashed lines) while others varied (peaks ‘a’ indicated by shorter arrows) (Figure 6.3). This may be due to errors from sampling as well as mixing.

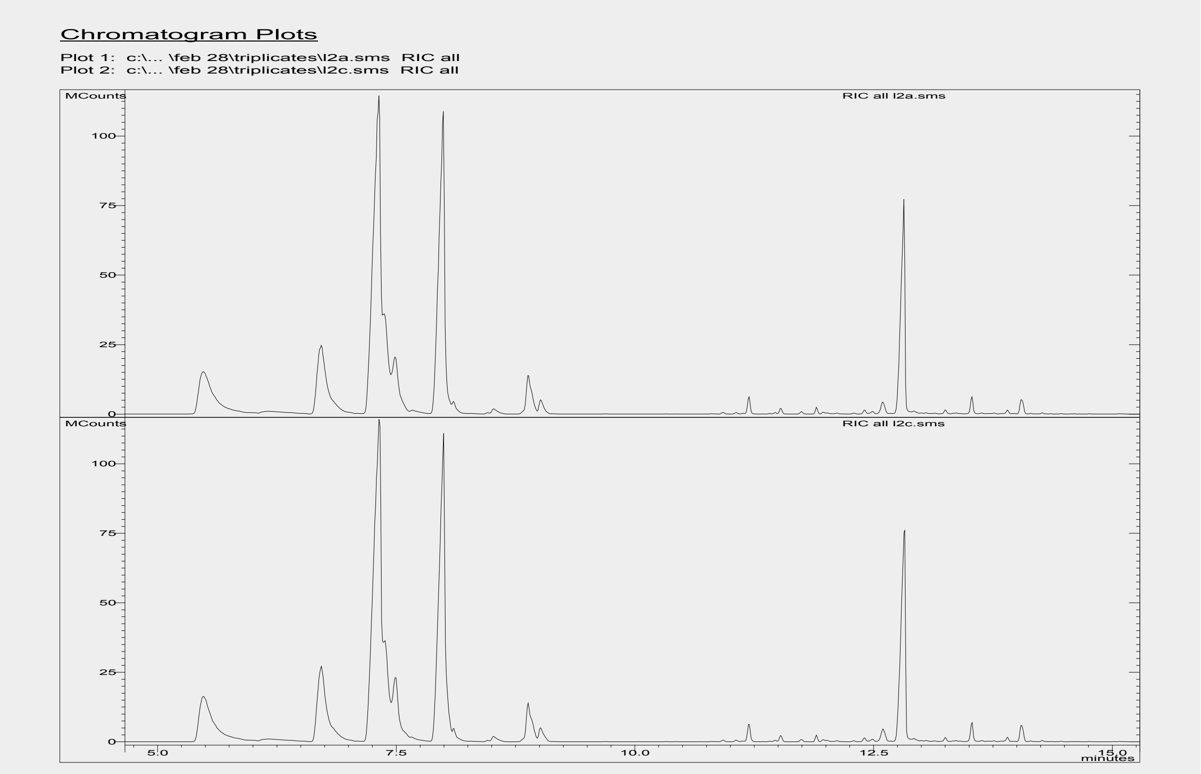


Figure 6.2 Two chromatograms from duplicate experiments stacked.

The plots chosen for drawing the trends for various treatments were obtained by choosing two close profiles out of each triplicate. The variations seen in the GC-MS profiles even after strictly adhering to the operation procedure for each experiment, is due to sources of error beyond that of manual control and fine-tuning.

Scope for improvement and the sources of error are discussed in the next section. The high degree of accuracy that is possible in such experiments can be enhanced by avoiding any source of error mainly in terms of microwave absorbers present in the rotational module. The materials that are transparent to microwaves such as Teflon are expensive but highly imperative in microwave equipment design for accurate results.

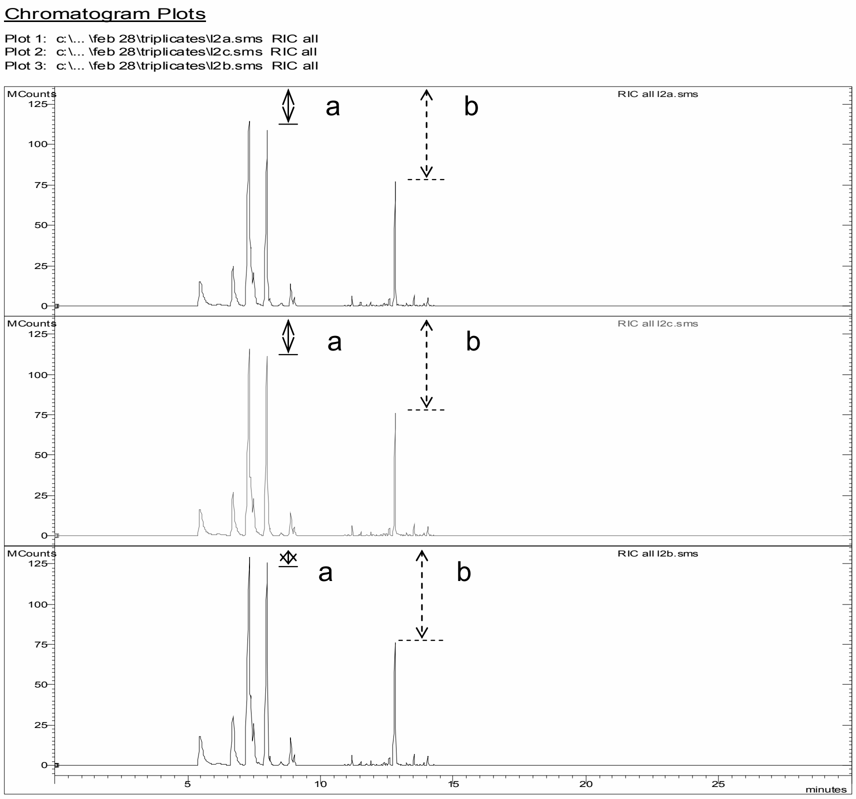


Figure 6.3 Triplicate chromatograms showing similarity and difference.

6.1.1 Frequency measurement of the microwave oven

The microwave oven was non-intrusively sensed using a loop probe and the frequency was measured using HP signal analyzer. The instrument had a resolution 100 Hz (instrument can give a resolution of 10 Hz with finer generator tuning). The measurement was made five times and the concordant value of 2.46 GHz was photographed using high-resolution camera since the digital export function of the analyzer was nonfunctional. The frequency was arrived at, after making assumptions on possible sources of error affecting the accuracy.

Figure 6.4 shows the cable and signal analyzer out of the microwave field influence. But in reality there are enough fields in measurement area to influence the value measured. In addition, the field may interact with the metal shield of the instrument, causing the scattering field from it as well as other metallic objects in the surrounding, to interfere with measured field. The cable had a RF shield and hence it could be assumed to have been insulated to a significant extent and that the coupling or pick-up of original signals occurred only at the loop antenna.

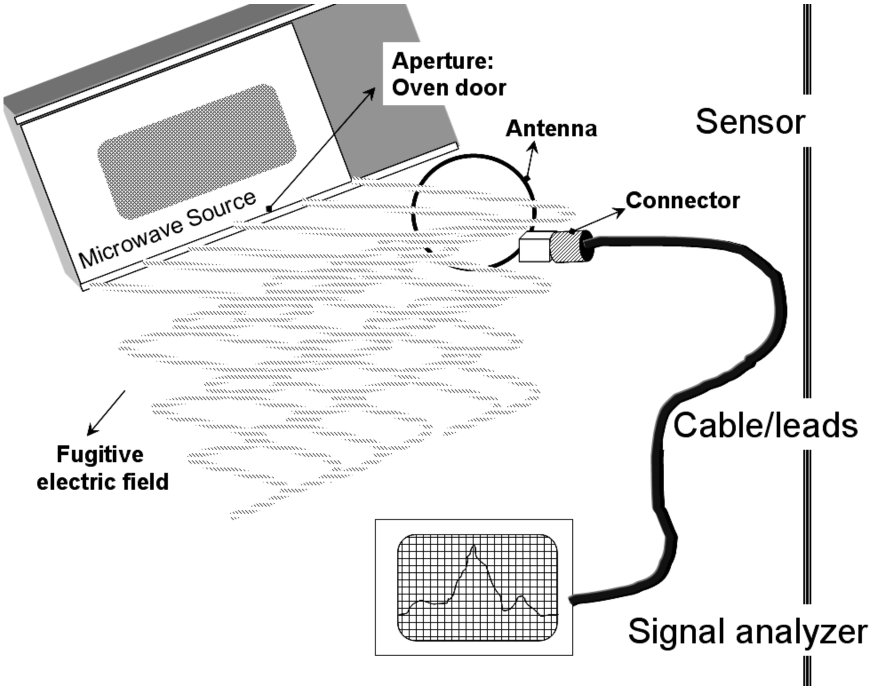


Figure 6.4 Sensing the microwave for frequency measurement.

Initial measurement was made with a span of 10 GHz (centering at 5 GHz) in the HP signal analyzer to find out if the oven was operating around 915 MHz or 2.45 GHz. Figure 6.5 shows the signal analyzer output for the coarse measurement showing the peak frequency at about 2.45 GHz for the microwave source used in the experiment.

The finer measurement with a span of 100 MHz shown in Figure 6.5 and 5.8 indicated the frequency as 2.460 GHz with 1 MHz resolution (each x-axis division in

Figure 6.5 is 0.492 GHz). The instrument was calibrated and handled by experienced technologists from Electrical Engineering department.

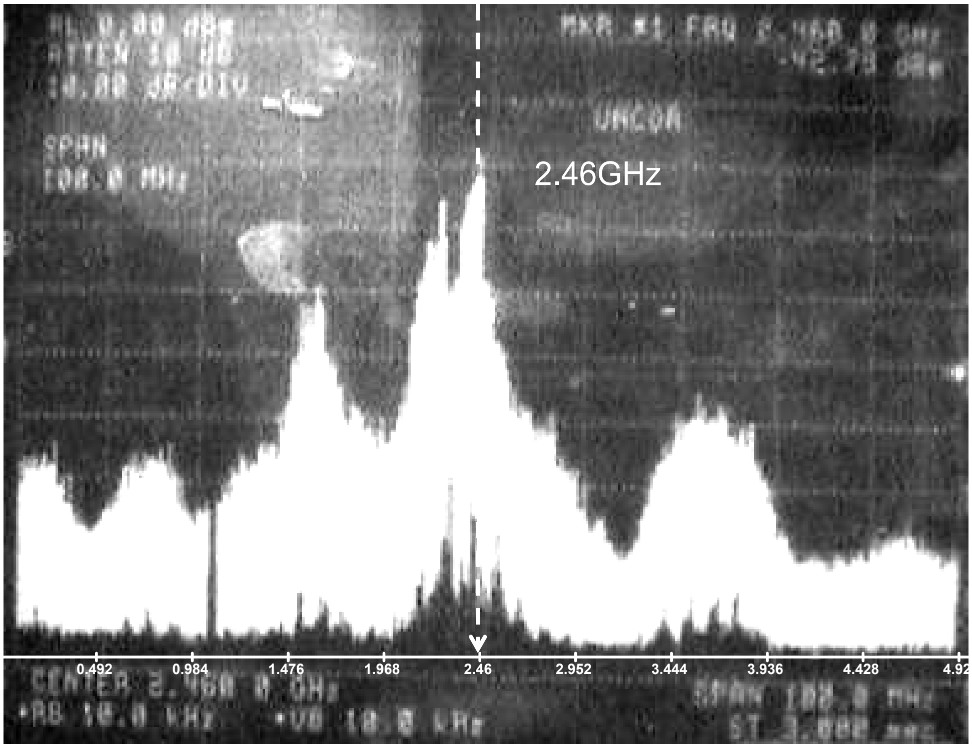
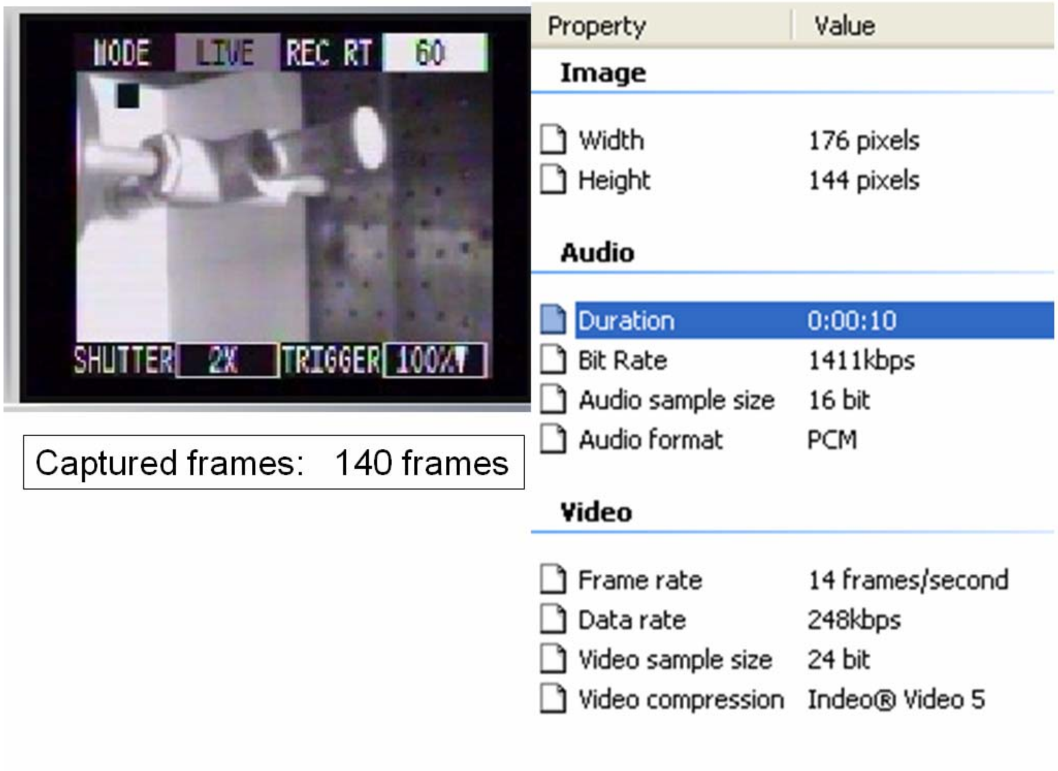


Figure 6.5 Signal analyzer output for the finer frequency measurement.

6.1.2 Standardization of speed of rotation

The rotational module consisted of an analog voltage regulator (Kepco power supply JQE 0 -15 V; 0 – 6 A), a DC step motor (Zenith 141-210 model: 1970040), ampoule holders and two glass ampoules (length 110.91 mm and diameter 28.56 mm). The choice of the desirable speed of rotation was arrived at by monitoring the particulate behavior of spice powder and recording it using a high-speed camera. Figure 6.6 shows the video clip parameters of the output from high speed camera Motionmeter© used in the current research to study the flow and thin layer characteristics of the spice powder in the rotating ampoule.



MODE LIVE REC RT 60

SHUTTER 2X TRIGGER 100%

Captured frames: 140 frames

Property	Value
Image	
Width	176 pixels
Height	144 pixels
Audio	
Duration	0:00:10
Bit Rate	1411kbps
Audio sample size	16 bit
Audio format	PCM
Video	
Frame rate	14 frames/second
Data rate	248kbps
Video sample size	24 bit
Video compression	Indeo® Video 5

Figure 6.6 The high speed camera video clip parameters.

The Zenith DC step motor (Figure 6.7) was used for its ease of speed to torque controllability. The speed could be brought down very close to zero. The motor was controlled using Kepco analog voltmeter. The torque was conveyed through a natural fiber belt to achieve a low uniform speed of rotation of the glass ampoule.

Over any period of operation due to wear, the belt tends to slip and the motor may convey non-uniform torque to the extraction unit. Further, the flow of spice particles which were assumed to be smooth and uniform every time, may only be a rough assumption. The influence of these effects was minimized by constant monitoring and maintenance of the rotational module. However, these are definitely regarded as potential sources of error. The rotational speed was stated as 5 rpm but its precision and accuracy had to be considered in the light of above variations. The rotation can be stated to have an accuracy of ± 5%.

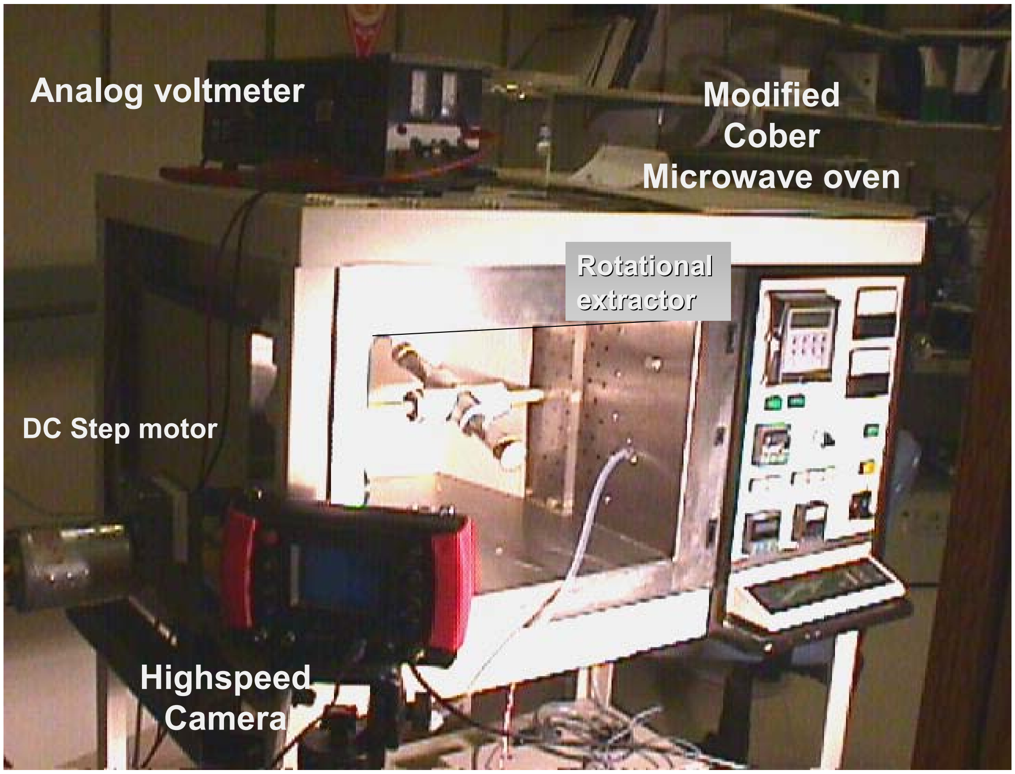


Figure 6.7 The rotational module of the experiment.

The speed of rotation was optimized so as to get the maximum duration of thin layer spread during the tumbling motion and for the smoothest flow of spice particles, assuming the particles have a reasonably predictable behavior. The speed of rotation was analyzed on video recordings made using Motionmeter© high-speed camera. Eight different speeds were analyzed for thin layer spread as well as flowability as shown in Table 6.1. In terms of accuracy, it was however a coarse measurement. The particulate flow and rotational effects on the particles, towards mixing and thin layer spreading, certainly need a more advanced study. Since the purpose of rotational experiments were to find a range of rotational speed with high thin layer duration and hold it constant in the current experiment, initial values were reliable under current experimental condition.

The speed of rotation where the thin layer spread as well as the flow were optimum, was found to be about 6 volts for the step motor or 5 rpm. The duration for which the spice particles spread as thin layer inside the ampoule was about 4.35 s. This rotational speed of the ampoule was held constant throughout the experiments.

Table 6.1 The rotational speeds of the extractor and related performance.

Motor voltage (volts)	Duration per rotation (seconds)	Particulate Flow (relative)	Thin layer duration (angle)	Thin layer duration (seconds)	comments
5	15.9	fair	90^0	4.35	Flow is not smooth
6	10.98	good	$<90^0$	<4.35	Smooth flow
7	8.58	good	60^0	1.56	Smooth flow
8	7.5	good	$<60^0$	<1.56	Spread is poor
10	5.28	good	30^0	0.44	Very Poor spread
11	4.78	good	$<30^0$	<0.44	Very Poor spread
13	3.98	good	$<15^0$	<0.166	No spread
16	3.46	good	$<15^0$	<0.166	No spread

Some spice particulates were seen at times adhering to the wall and affecting the flow. The overall flow however remained unaffected most of the time. It is important to note the extent of such effects have been completely neglected in the current study assuming the high randomness of the event.

6.1.3 Microwave property of standard reference (water)

The dielectric loss of water at the operating frequency of the microwave oven is used in the microwave field calculation at the location of the extraction. Although the value is reported in the literature for pure water, it was imperative to confirm the value for the water used in the current experiment so as to take into account the aberration caused by ionic impurities.

6.1.3.1 Dielectric loss of water at 2.46 GHz

The dielectric loss of water at 2.46 GHz was measured using a coaxial probe connected to a HP Vector Network Analyzer.

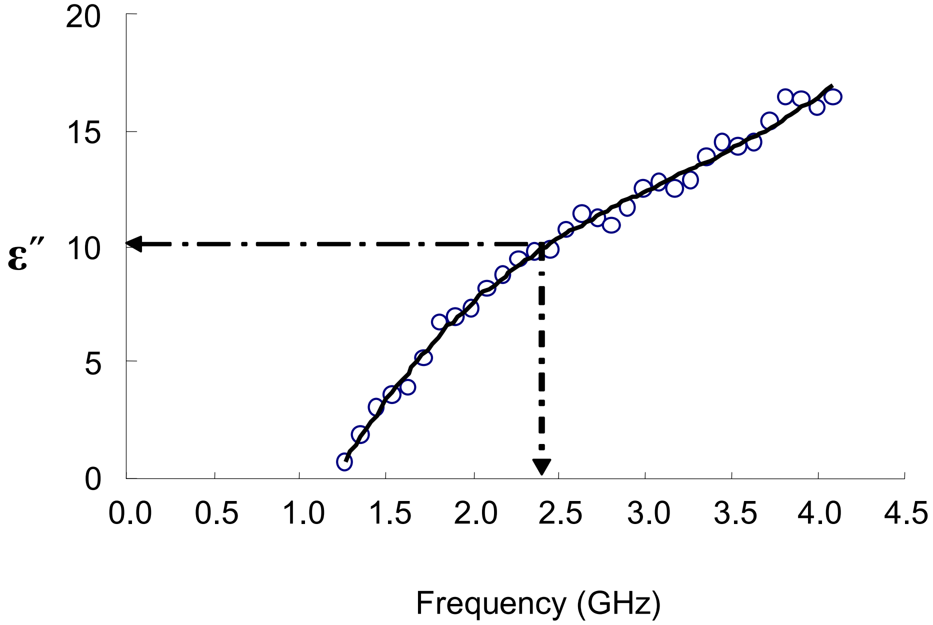


Figure 6.8 Relationship of dielectric loss factor ε″ of water with the frequency of COBER™ microwave.

The HP Vector Network Analyzer was calibrated to second decimal place. The value obtained was compared with the literature. Since the published literature gave exactly the same value, the measured dielectric loss was considered reliable. The result of the measurement is displayed in the plot of dielectric property of water against the frequency of microwave in Figure 6.8. At 2.46 GHz the dielectric loss of water was found to be 10.

The real versus imaginary part of dielectric permittivity are plotted in the literature for a range of frequencies. These plots are called Cole-Cole plots. The measured dielectric property was plotted as Cole-Cole plot with a polynomial fit and overlaid on a similar plot published (Jurgen 2003) in the literature as shown in Figure 6.9. The two plots were found to be congruent.

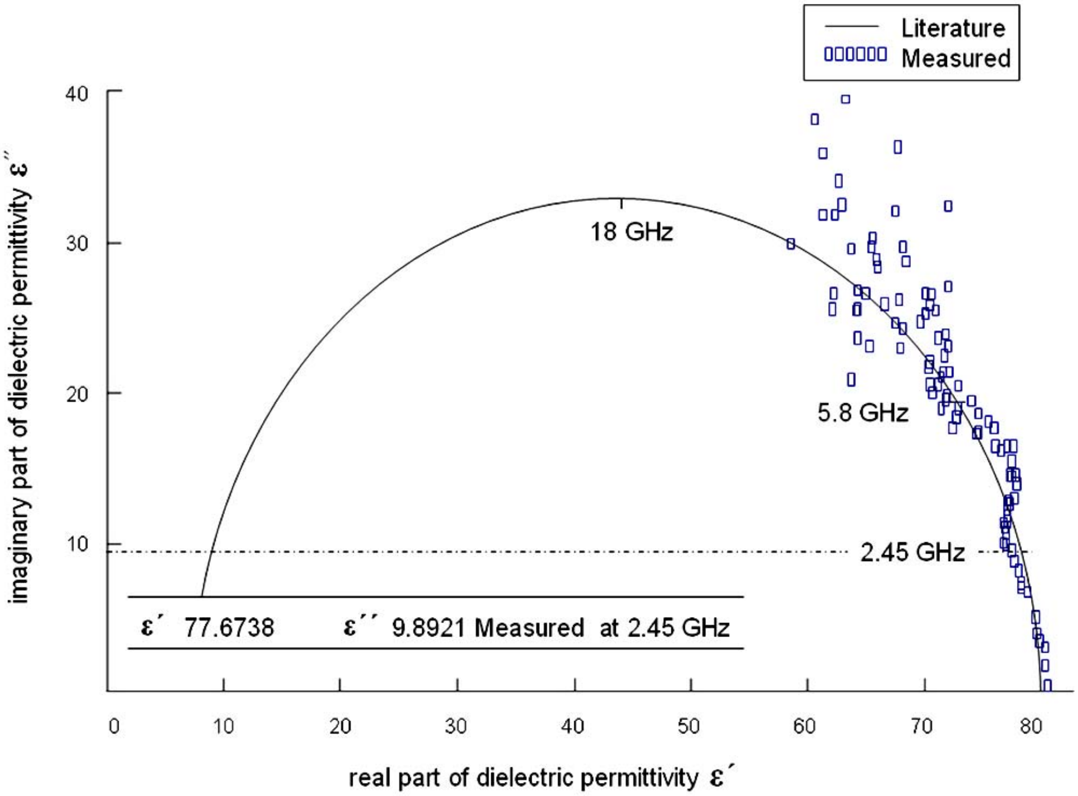


Figure 6.9 The Cole-Cole plot for water.

6.1.3.2 Penetration depth of microwaves in water at 2.46 GHz

The penetration depth or the depth at which the microwave power reduces to about 37% after being absorbed in a dielectric material is given by the following equation

$$Dp \approx \frac{\lambda_0}{2\pi} \times \frac{\sqrt{\varepsilon'}}{\varepsilon''} \tag{6.1}$$

Dp penetration depth measured in cm

λo wavelength under vacuum measured in cm

ε' dielectric constant

ε" dielectric loss

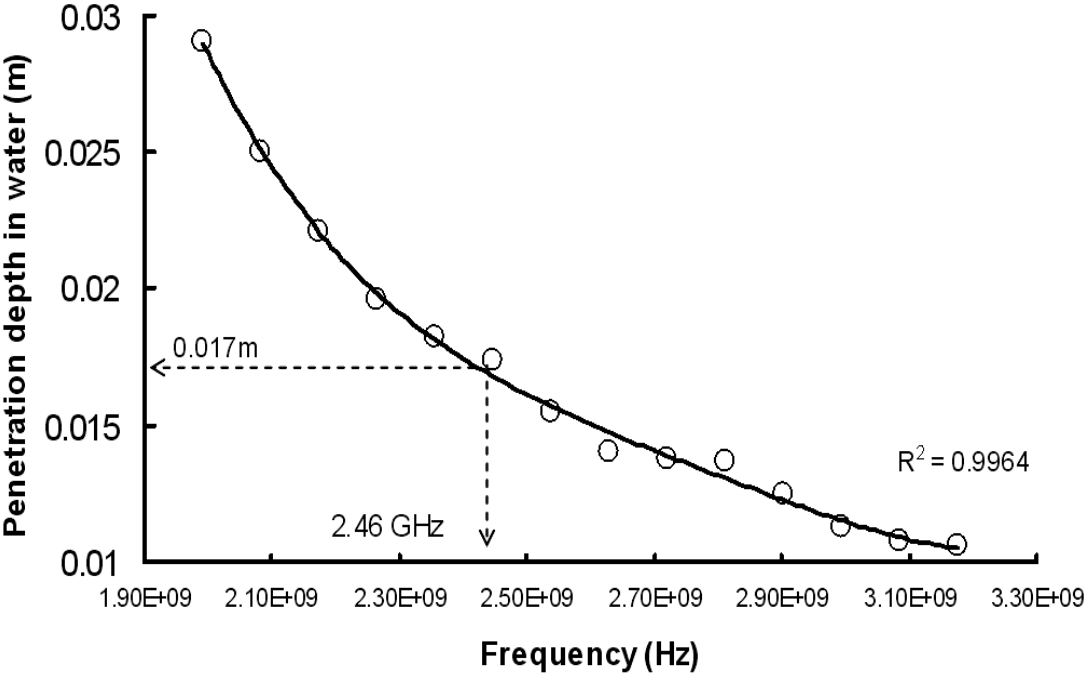


Figure 6.10 The plot of penetration depth as it varies with frequency of the microwave.

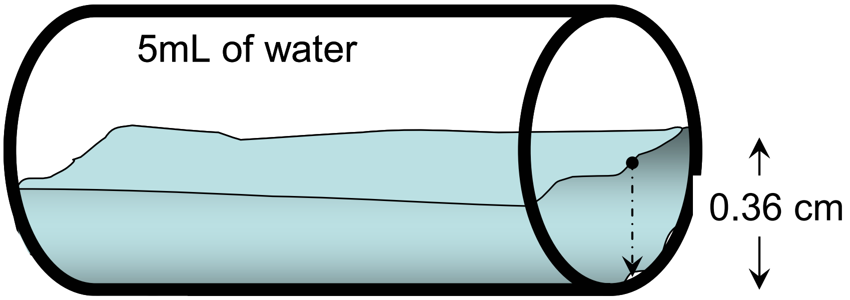


Figure 6.11 Volume of water in horizontal ampoule.

A table on the penetration depth based on measured dielectric values is given in Appendix E. The plot in Figure 6.10, gives the penetration depth of water at 2.46 GHz as 1.7 cm (or 40 mL in the ampoule). This is the value that is used for arriving at the optimum volume of water as a reference used in the determination of electric field inside the microwave oven. If the penetration depth has to be as low as 0.36 cm (less than 25% of the estimated value in order to be unaffected by field attenuation under real conditions), the volume that could be taken in the ampoule was arrived at using the equation 6.2 . The volume of water in a horizontal ampoule V is given by:

$$V = L[(r^2 a\cos\frac{(r-h)}{r}) - ((r-h)(2rh-h^2)^{\frac{1}{2}})] \qquad (6.2)$$

L = Length of the ampoule 11.09cm
h = depth of water in horizontal position 0.36 cm
r = radius of the ampoule 1.33 cm
V = calculated volume of water 4.99 cm^3
acos= $\cos^{-1}$

Figure 6.12a shows the depth of water in the ampoule as it forms a thin layer in the horizontal position. Although 1.7 cm is the penetration depth of water at 2.46 GHz, it can be estimated that in the presence of other absorbers such as PVC I and glass present in the rotatory set-up, the penetration depth would be even lower.

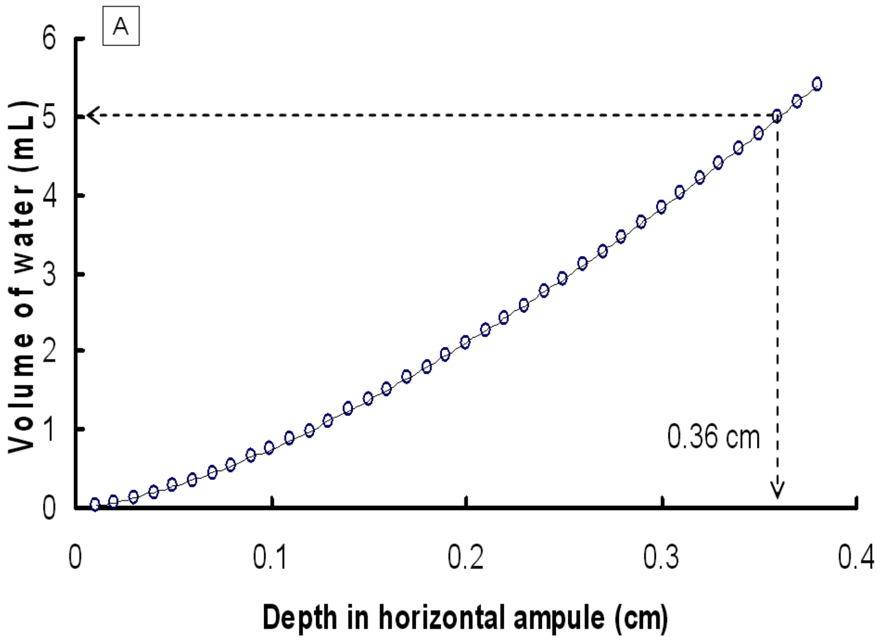


Figure 6.12a Plot showing volume variation as a function of depth of water in ampoule.

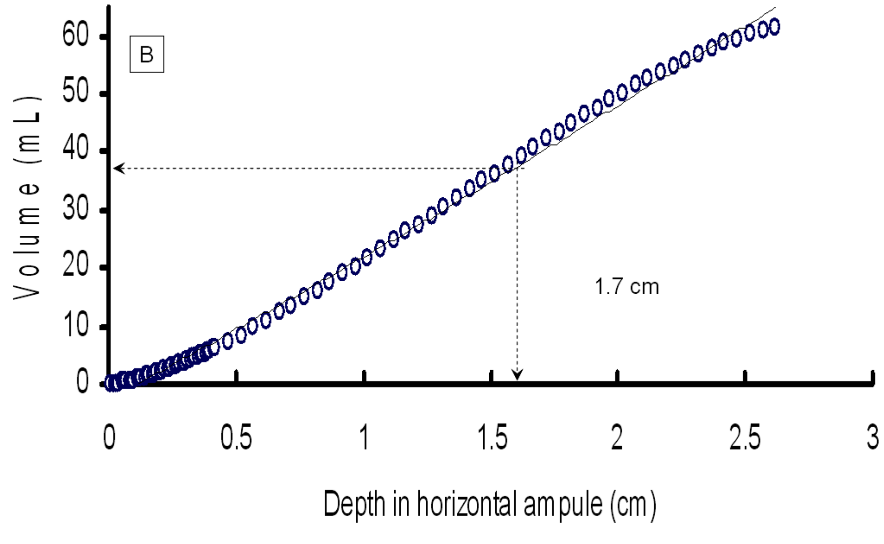


Figure 6.12b Volume of water in ampoule corresponding to penetration depth.

The real penetration depth could be close to half the calculated value. In order to ensure total penetration of microwaves into the reference ampoule containing water, it is imperative that the depth of water in the ampoule at horizontal position be sufficiently lower than penetration depth estimated under ideal conditions. Hence 0.36 cm, about 25% of the calculated penetration depth was used as the measure for the volume of water in the ampoule. At 0.36 cm the volume rounds off to 5 mL of water which would lead to a very low attenuation. The volume corresponding to the penetration depth of microwave Dp (where significant attenuation would occur) is 40 mL of water in the ampoule. Figure 6.12b shows the volume of water in the ampoule corresponding to a depth of 1.7 cm which is the penetration depth of water at 2.46 GHz.

6.1.4 Infrared temperature measurements

The temperature rise in a microwave process is difficult to measure owing to the sensitive nature of microwaves. Fiber optic probes are usually employed or alternatively, IR thermometry or thermography is used. Fiber optics come with rotational slip-ring joints with multiple passes so as to effect rotating interfacial measurements. Thermography is a high-end application of IR temperature measurements. At the lower end (cheaper, user friendly and reasonably accurate) is the IR thermometer (or pyrometer as it is sometimes referred to).

In the current research, a wide range infrared thermometer 42530 from Extech Instruments Corporation was used. This was easy to use for a rotational module that had to take positional variation after each experiment. The instrument is expected to have high reliability under ideal conditions with a range of -50 to 538°C, accuracy of ±2% of reading or ±4°F/ 2°C and a resolution of 0.1°F/°C.

However, there are specified ideal conditions which are as follows.

(1) Field of view: 8:1 (at 8" distance measure 1" target). For an ampoule of about 1" diameter, the accuracy depends on maintaining the IR thermometer no farther than 8".

(2) The accuracy is low for reflective and through transparent surfaces

The temperature difference calculation however removes any systemically occurring errors and hence for the present experiment involving measurement of temperature rise in a glass ampoule, the precision was reliable, ranging at ±1.7 ^{0}C leading to an accuracy in the microwave field measurement to a first decimal place.

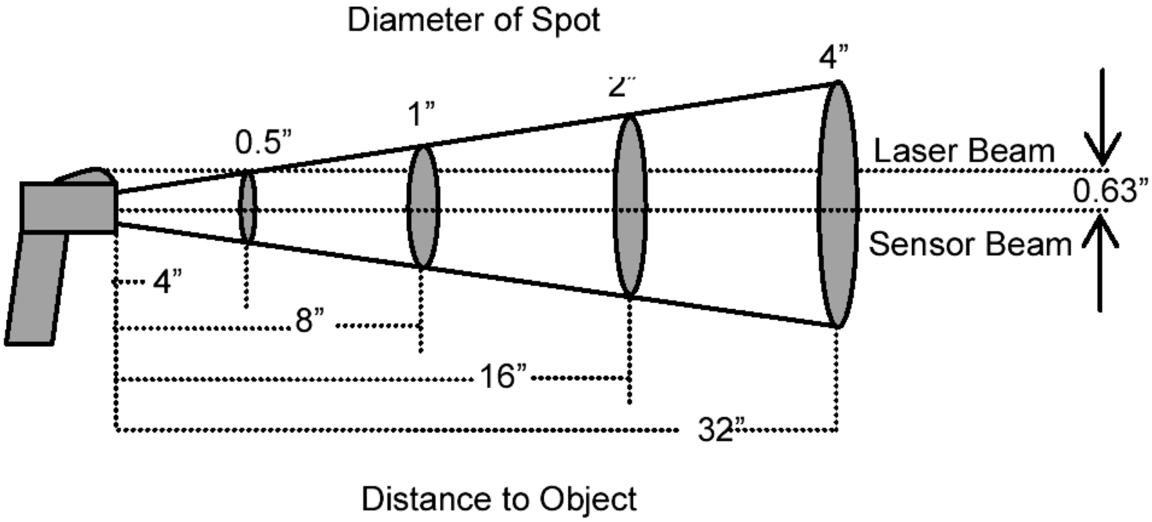


Figure 6.13 The focus object in infrared thermometry.

6.1.5 Establishing the microwave field at positions inside oven cavity

The temperature rise of the reference (water) at each position on the slider was used to calculate the RMS electric field strength in volts per meter. The basic assumption made here is that the microwave electric field is absorbed exclusively by water reference causing it to heat. This, however, is a large approximation considering the various marginal absorbers present in the oven cavity. The wheels, axel and ampoule holder are made from PVC I (Schedule 40 D2466-88). Borosilicate is the material of the glass ampoule. There are two metallic screws in the rotational module present at the periphery of oven cavity. Invariably they absorb a portion of electric field causing attenuation of the field within the cavity.

Further errors are to be accounted for in the purity of water. Dielectric loss characterizes the loss contributions from various absorption mechanisms of water molecules in the presence of applied field. Dielectric loss value is a good indicator of the extent to which applied microwave is converted to thermal energy by a dielectric substance. By measuring the temperature rise, it is possible to estimate the electric field in the region of microwave exposure if dielectric loss, the frequency and specific heat of the dielectric are known. But, it is to be recognized that with even minor inclusion of ionic impurities (for example from fingerprints), the dielectric loss rises sharply. The degree of error in the estimation of energy absorbed in the ampoule containing the reference needs to studied in detail using more sophisticated instrumentation than those used in the current research.

However, the purpose of the experiment is to establish the relative strengths of the electric field so as to study its relative effects on the extraction of essential oils. Hence the accuracy of the RMS electric field strength calculated though not accurate yields the cavity profile with a precision in terms of relative electric field strength.

The set of equations used for the RMS electric field strength calculation is described below. The heat equation is used to derive the value of electric field. Heat generated is given by equation 6.2:

$$Q = mc\Delta TF \qquad (6.2)$$

Where:
Q = the heat generated in joules;
c = the specific heat (kJ kg^{-1} K^{-1});
m = the mass (kg); ***T*** = the measured temperature (K);
F = an empirical factor that accounts for heat from other sources. This was assumed to be 1 in the present experiment for simplification of the calculation. There is scope for understanding the full implication of this factor.

Since microwave power can be stated in terms of heat as shown in equation 6.4 and 6.5, it is now possible to evaluate electric field from temperature measurements in a microwave process.

$$Q = \Delta t P \tag{6.4}$$

$$Q = \Delta t \omega \varepsilon_o \varepsilon'' E_{rms}^2 V \tag{6.5}$$

Where:

Δ ***t*** = time of microwave exposure in seconds;

P = average power in watts;

ω = angular frequency (rad. s^{-1});

ε_0 = the permittivity in free space (F m^{-1});

ε'' = effective dielectric loss;

V = the volume of substrate that is exposed to microwaves (m^3) and

E_{rms}^2 = the square of effective electric field (V/m).

6.1.5.1 Electric field estimation within the oven cavity

The electric field $\boldsymbol{E}_{\mathrm{rms}}$ obtained by substituting the values in equation 6.5 was then tabulated as summarized in in Table 6.2.

Table 6.2 Summary of microwave field at different labeled positions.

Position Label	Field (V/m)	Location (mm)
R0	81.84	15.62
R1	83.19	25.62
R2	87.41	35.62
R3	86.83	50.62
R4	93.46	65.62
R5	92.14	80.62
MR	85.32	95.62
ML	84.56	171.78
L2	81.83	180.8
L1	81.1	189.8
L0	58.64	198.95

Table 6.3 The electric field calculations inside the oven cavity from heat equation.

Expt id	Location (mm)	ΔT	Δt	Heat (J)	E^2	E
L0a	199	8.1	30	169.3	3739.4	61
L0b	199	8.2	30	171.4	3785.5	62
L0c	199	5.5	30	115	2539.1	50
L0d	200	8.2	30	171.4	3784.7	61.5
R0a	15.6	16.1	30	336.6	7432.6	86
R0b	15.6	13.6	30	284.3	6278.5	79
R0c	15.6	14.2	30	296.9	6555.5	81
R0d	16.6	14.2	30	296.8	6554.5	81
Mra	95.6	15.6	30	326.1	7201.8	85
Mrb	95.6	15.5	30	324	7155.6	85
Mrc	95.6	15.1	30	315.7	6970.9	83
Mrd	95.6	16.9	30	353.3	7801.9	88
Mre	95.6	16.9	30	353.2	7800.4	88.3
Mla	171.8	16.6	30	347	7663.4	88
Mlb	171.8	15.6	30	326.1	7201.8	85
Mlc	171.8	14.2	30	296.9	6555.5	81
Mld	171.8	15.6	30	292.6	6462.6	80.4
L2a	180.8	14	30	292.7	6463.1	80
L2b	180.8	13.7	30	286.4	6324.6	80
L2c	180.8	16.4	30	342.8	7571.1	87
L2d	180.8	14	30	292.6	6462.6	80.4
L1a	189.8	14.1	30	294.8	6509.3	81
L1b	189.8	14	30	292.7	6463.1	80
L1c	189.8	14.8	30	309.4	6832.4	83
L1d	189.8	14.1	30	294.8	6509.3	80.7
R1a	25.6	15.3	30	319.9	7063.3	84
R1b	25.6	13.8	30	288.5	6370.8	80
R1c	25.6	15.2	30	317.8	7017.1	84
R1d	25.6	15.7	30	328.2	7247.9	85
R1e	25.6	15.7	30	328.2	7247.1	85.1
R2a	35.6	16.8	30	351.2	7755.8	88
R2b	35.6	16.6	30	347	7663.4	88
R2c	35.6	16.2	30	338.7	7478.8	86
R2d	35.6	16.6	30	347	7663.3	87.5
R3a	50.6	18.2	30	380.5	8402.1	92
R3b	50.6	15.4	30	321.9	7109.4	84
R3c	50.6	15.9	30	332.4	7340.3	86
R3d	50.6	15.9	30	332.3	7339.3	85.7

Field was plotted against position to get the graph shown in Figure 6.14. The calculated values are given in Table 6.3.

Table 6.3 (continued): The electric field calculations inside the oven cavity from heat equation.

Expt id	Location (mm)	ΔT	Δt	Heat (J)	E^2	E
R4a	65.6	18.5	30	386.7	8540.6	92.0
R4b	65.6	20	30	418.1	9233	96.0
R4c	65.6	18.6	30	388.8	8586.7	93.0
R4d	65.6	18.6	30	388.8	8585.9	92.7
R5a	80.6	17.3	30	361.7	7986.6	89.0
R5b	80.6	19.5	30	407.7	9002.2	95.0
R5c	80.6	18.4	30	384.7	8494.4	92.0
R5d	80.6	18.4	30	384.6	8493.5	92.2

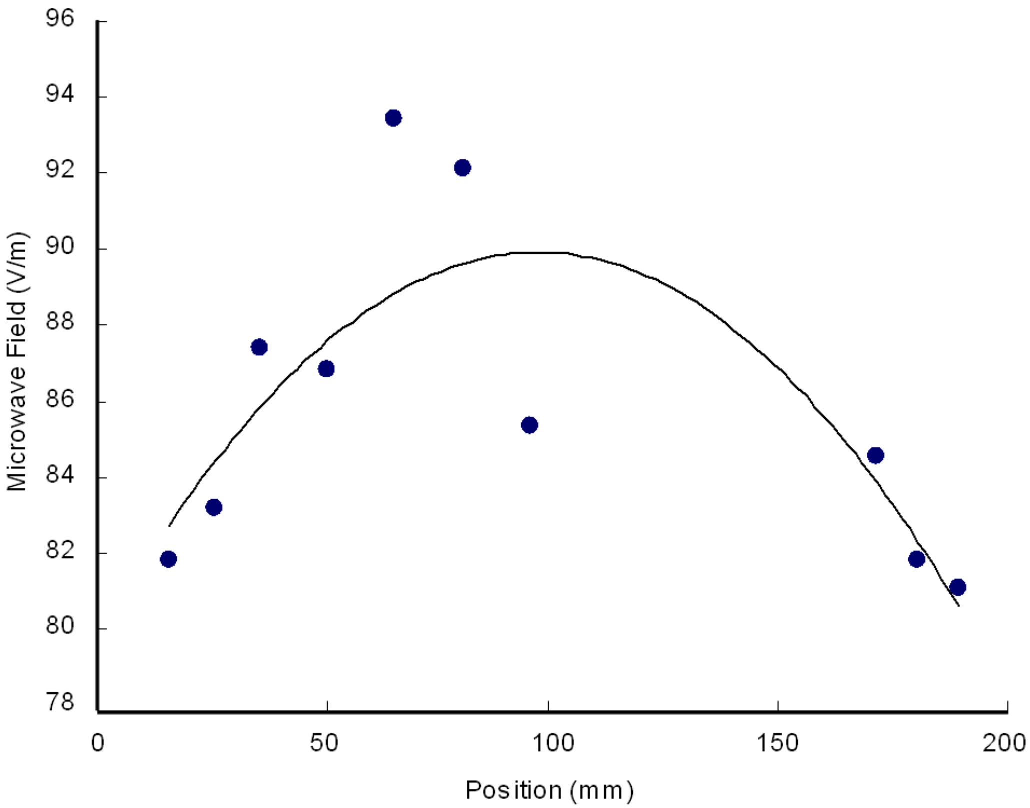
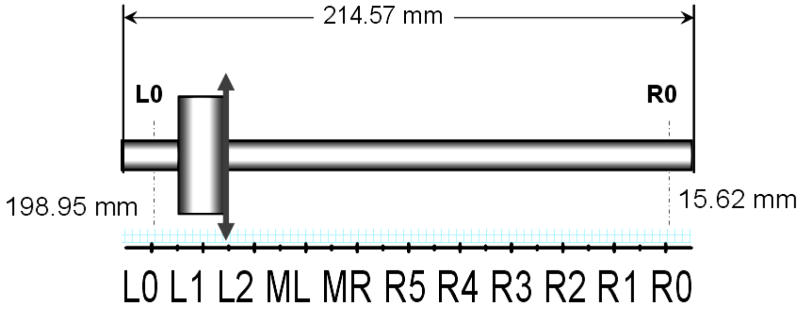


Figure 6.14 The microwave oven cavity profile in terms of electric field distribution with respect to position (mm from the right extreme of oven cavity).



R0 [**81.84 V/m**] *is located* 15.62 mm *from the cavity wall at right extreme*

R1[**83.19 V/m**] is at 25.62 mm
R2[**87.41 V/m**] 35.62 mm
R3[**86.83 V/m**] 50.62 mm
R4[**93.46 V/m**] 65.62 mm
R5[**92.14 V/m**] 80.62 mm

MR[**85.32 V/m**] 95.62 mm
ML[**84.56 V/m**] 171.78 mm
L2 [**81.83 V/m**] 180.80 mm
L1 [**81.10 V/m**] 189.80 mm
L0 [**58.64 V/m**] 198.95 mm

POSITION SYMBOL [**MICROWAVE FIELD V/m**] LOCATION (**mm**) FROM RIGHT

Figure 6.15 Cavity positions with their respective microwave field values.

6.2 Standardization of Essential Oil Extraction, Sampling and Analysis.

The extraction, sampling and analysis were performed initially on a single weighment of the sample by repeating several cycles upon one sample. This gives an indication of the extent to which ampoule is gas tight after each injection for SPME.

6.2.1 Effect of repeated extraction and analysis on a given sample.

Three set of experiments as discussed in 6.2.2, 6.2.3, 6.2.4, were performed on black pepper to see if a single sample (single sampling and weighment) can be used for studying cumulative effects of repeated extraction.

Table 6.4 The repeated extraction with its estimated cumulative value.

Duration of exposure (s)	Simulated cumulative value (ion counts)
23	23
63	86
123	209
203	412
283	695

For cumulative effect the plot would be expected to give the trend shown below in Table 6.4. The table shows a simple relationship of a series of duration lengths with its corresponding accumulated value on successive additions. This would resemble an exponential curve. The effect of successive extraction and analysis seen in the experiment showed a response far from this exponential trend.

A discussion on repeated extraction and analysis on a single weighment is given at the end of section 6.2.5. Following sections (6.2.2 to 6.2.5) are results of the four experiments to study repeated use of an ampoule weighment.

The repeated analysis is a standardization experiment to see if a single weighment of sample can yield results for a range of extractions. If it were possible, extraction trends could be obtained with minimal errors in sample weight as well as other characteristics. Repeat experiment was performed in right, left and mid region of the microwave oven.

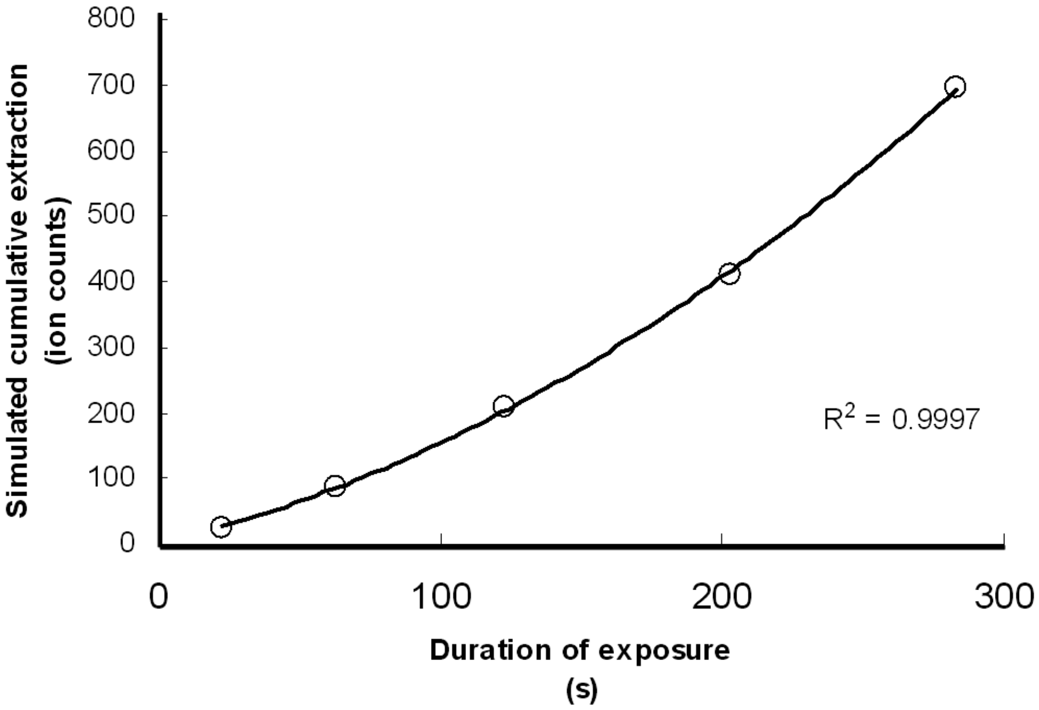


Figure 6.16 Successive extraction, and analysis on a given sample.

6.2.2 Repeated extraction and analysis on a given sample at R0

R0 is the position in the right extreme of the slider inside microwave oven. A duplicate repeat experiment was performed at R0 with increment in exposure time on a single sample. Table 6.5 gives the extraction in terms of cumulative ion counts. Figure 6.17 gives the trend as a lateral plot where as cumulative effect would be expected to give a curve such as the one shown in Figure 6.16.

All the plots of the standardization experiment were compared as linear fits but due to lack of trend (a low R^2 value), these plots are shown as scatter plots.

Table.6.5 Repeated extraction and analysis on a given sample at R0.

SI No	Expt Name	Location (mm)	Field (V/m)	Duration (s)	Extraction (ion counts)
1	MR0a	15.62	82	23	7.50×10^7
2	MR0b	15.62	82	63	7.98×10^7
3	MR0c	15.62	82	123	7.71×10^7
4	MR0d	15.62	82	203	8.98×10^7
5	MR0e	15.62	82	283	8.65×10^7
7	M3R0a	15.62	82	23	7.98×10^7
8	M3R0b	15.62	82	63	9.90×10^7
9	M3R0c	15.62	82	123	9.21×10^7
10	M3R0d	15.62	82	203	9.54×10^7
11	M3R0e	15.62	82	283	9.07×10^7

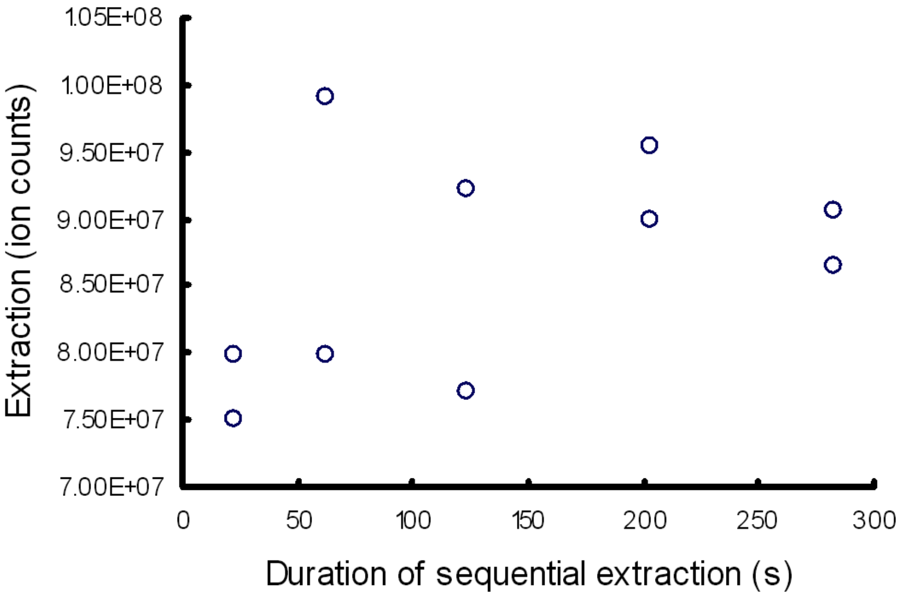


Figure 6.17 Effect of repeated extraction and analysis on a given sample at location R0.

6.2.3 Repeated extraction and analysis on a given sample at L0

L0 is the position in the left extreme of the slider inside the microwave oven. A duplicate repeat experiment was performed at L0 similar to that done at R0 with increment in exposure time on a single sample. Table 6.6 gives the details of the experiment with extraction in terms of cumulative ion counts. Figure 6.18 gives trend for repeated extraction at position L0 (cf. the expected trend in Figure 6.16).

Table.6.6 Repeated extraction and analysis on a given sample at L0.

Sl No	Expt Name	Location (mm)	Field (V/m)	Duration (s)	Extraction (ion counts)
1	ML0a	198.95	59	23	1.30×10^{8}
2	ML0b	198.95	59	63	8.59×10^{7}
3	ML0c	198.95	59	123	9.36×10^{7}
4	ML0d	198.95	59	203	9.42×10^{7}
5	ML0e	198.95	59	283	1.02×10^{8}
6	M2L0a	198.95	59	23	9.49×10^{7}
7	M3L0a	198.95	59	23	1.02×10^{8}
8	M3L0b	198.95	59	63	8.96×10^{7}
9	M3L0c	198.95	59	123	9.52×10^{7}
10	M3L0d	198.95	59	203	7.96×10^{7}

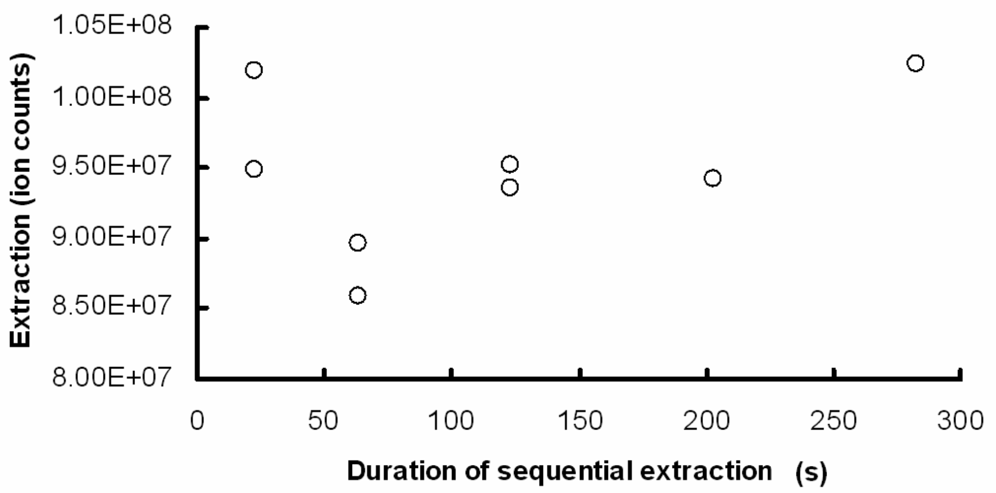


Figure 6.18 Effect of repeated extraction and analysis on a given sample at location L0.

6.2.4 Repeated extraction and analysis on a given moist sample at R5

R5 is the position close to the mid point of the slider inside microwave oven. A duplicate repeat experiment was performed at R5 similar to that done at R0 and L0 with increment in exposure time on a single sample. Since R5 also happens to be a region of high field as seen in Figure 6.14, it was felt appropriate to conduct an experiment with added water content so as to increase the temperature rise and see the outcome of repetition with higher temperature extraction on a single sample. Table 6.7 and Figure 6.19 show the trends for repetition at R5 position with water content. The water content inclusion is performed by adding drops of water then mixing until thoroughly mixed and weighing to required percentage. In the literature addition of water content to dry herbs to improve microwave processing has been referred to as rehydration.

Table.6.7 Repeated extraction and analysis on a given sample at location R5 at 15% water content.

Sl no	Expt Name	Location (mm)	Field (V/m)	Duration (s)	Extraction (ion counts)
1	M1R5a	80.62	92	23	1.01×10^8
2	M1R5b	80.62	92	46	9.63×10^7
3	M1R5c	80.62	92	109	7.18×10^7
4	M2R5a	80.62	92	23	1.04×10^8
5	M2R5b	80.62	92	46	9.09×10^7
6	M2R5c	80.62	92	109	1.02×10^8

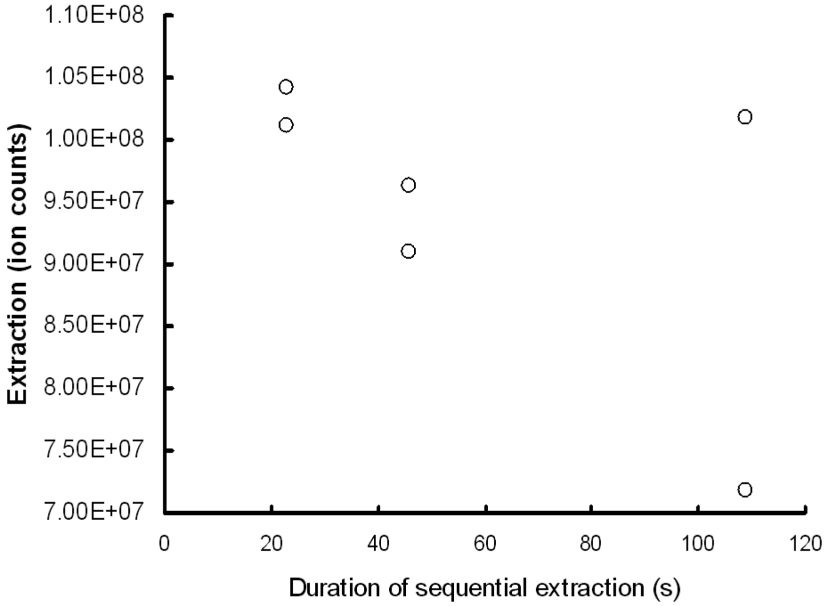


Figure 6.19 Effect of repeated extraction and analysis on a given sample at location R5 at 15% water content.

6.2.5 Repeated extraction and analysis on a given dry sample at R5

Table 6.8 and Figure 6.20 show the trends for repetition at R5 position in dry condition. Figures 6.17 to 6.20 clearly show lack of response compared to the expected trend shown in Figure 6.16. The initial experiments served to confirm the importance of independent weighment for each experiment even in a triplicate set.

Table.6.8 Effect of repeated extraction and analysis at R5 without rehydration.

Sl No	Expt name	Location (mm)	Field (V/m)	Duration(s)	Extraction (ion counts)
1	R5at10	80.62	92	10	5.05×10^9
2	R5at20	80.62	92	30	4.22×10^9
3	R5at60	80.62	92	90	5.08×10^9
4	R5at180	80.62	92	270	4.20×10^9

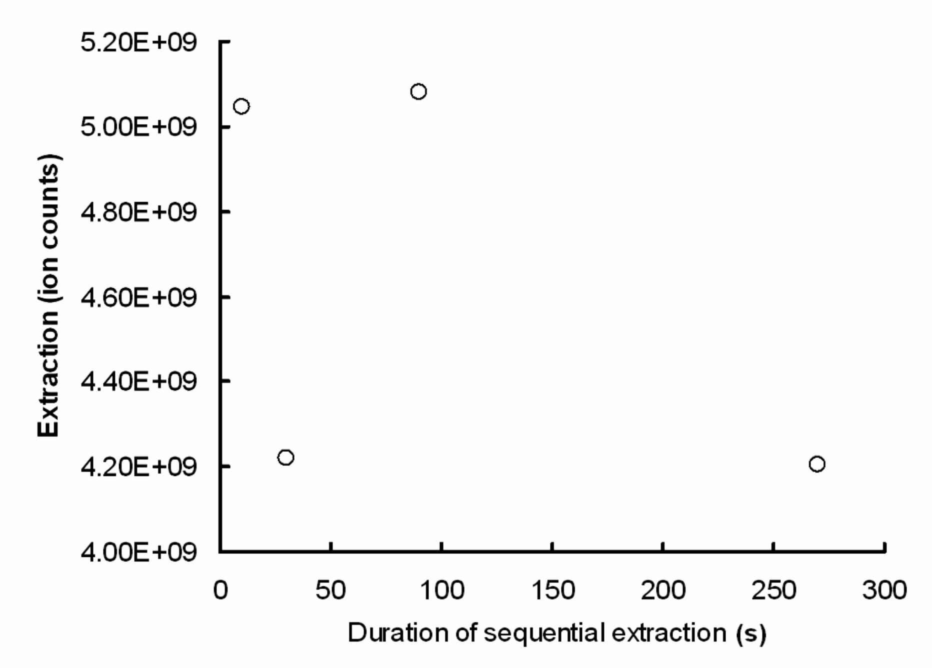


Figure 6.20 Effect of repeated extraction and analysis at location R5 in dry condition.

The independent weighment gives rise to errors in sampling the spice powder and weigment errors. But their effects have been minimized by greater attention to details and closely observing laboratory standard operating procedure.

The experimental results in 6.2.2 to 6.2.5 on a single sample (by repeatedly extracting and analysis by means of headspace SPME and GC-MS), showed that it is not feasible. It is neither possible to study trends on a single sample on cumulative effects of extraction, nor possible to study a simple triplicate using a single sample with three extractions.

The ANOVA conducted using SAS indicated high probability (Pr) value showing that the trends obtained in the repeat experiments did not reflect a definite effect of independent variable, the electric field upon the extraction and that the experiment was not feasible in its current form.

The SPME procedure involves puncturing of the septum - however close to being gas tight, the puncturing might be, in the sense of not releasing any volatiles out of the closed system of sampling - it has finite extent of leakage. This is especially felt in the subsequent runs of extraction. Thus, each experiment needs a fresh ampoule and septum (a washed and dried septum was found equally reliable).

Hence the extraction studies were conducted using fresh sample for each of the three triplicate experiments. The ampoule was washed and thoroughly dried in an oven at 120^0C for 45 min before each experiment. The septum was washed and dried in the open air. A hand held fan was used to accelerate the drying. The septum was pierced for SPME at different location every time so as to avoid a permanent leak. The material of the septum has the property of closing its pore immediately after piercing which is further enhanced on washing and drying before every use.

6.3 Effect of Microwave Field Variation on Extraction of Dry Pepper

The procedure to sense field effect on extraction was central to this work.

Table 6.9 The effect of microwave field on extraction of dry pepper.

Sl No	Location	Position (mm from right))	microwave Field (V/m)	Extraction (ion counts)
1	R0	15.62	82	4.57×10^9
2	R0	15.62	82	4.36×10^9
3	Mr	95.62	85	4.19×10^9
4	Mr	95.62	85	3.92×10^9
5	Ml	171.78	85	3.92×10^9
6	Ml	171.78	85	4.18×10^9
7	L2	180.8	82	3.99×10^9
8	L2	180.8	82	4.19×10^9
9	L1	189.8	81	3.29×10^9
10	L1	189.8	81	3.55×10^9
11	R1	25.62	83	4.82×10^9
12	R1	25.62	83	4.93×10^9
13	R2	35.62	87	4.4×10^9
14	R2	35.62	87	4.09×10^9
15	R3	50.62	87	5.09×10^9
16	R3	50.62	87	4.87×10^9
17	R4	65.62	93	4.99×10^9
18	R4	65.62	93	4.31×10^9
19	R5	80.62	92	5.05×10^9
20	R5	80.62	92	5.08×10^9

The field measurements were repeated at eleven field positions in the microwave for pepper and its extraction responses were compared and statistically analyzed using analysis of variance (ANOVA). Pepper powder responds to microwave extraction well and shows a well separated GC-MS as seen in Figure 6.21 which also has the major components of pepper essential oils labeled.

The effect of microwave field on extraction would be expected to be a direct proportionality relationship. But is it measurable is what has been in question since there has not been any reported method to establish this relationship. In Figures 6.22 and 6.23, pepper extraction versus microwave field is shown. It gives a clear trend for independent triplicate experiments. The accuracy of the trend can be significantly improved with improvization in the various instrumental measurement steps as well as by automation of the several manual steps (cf. steps given in section 6.1).

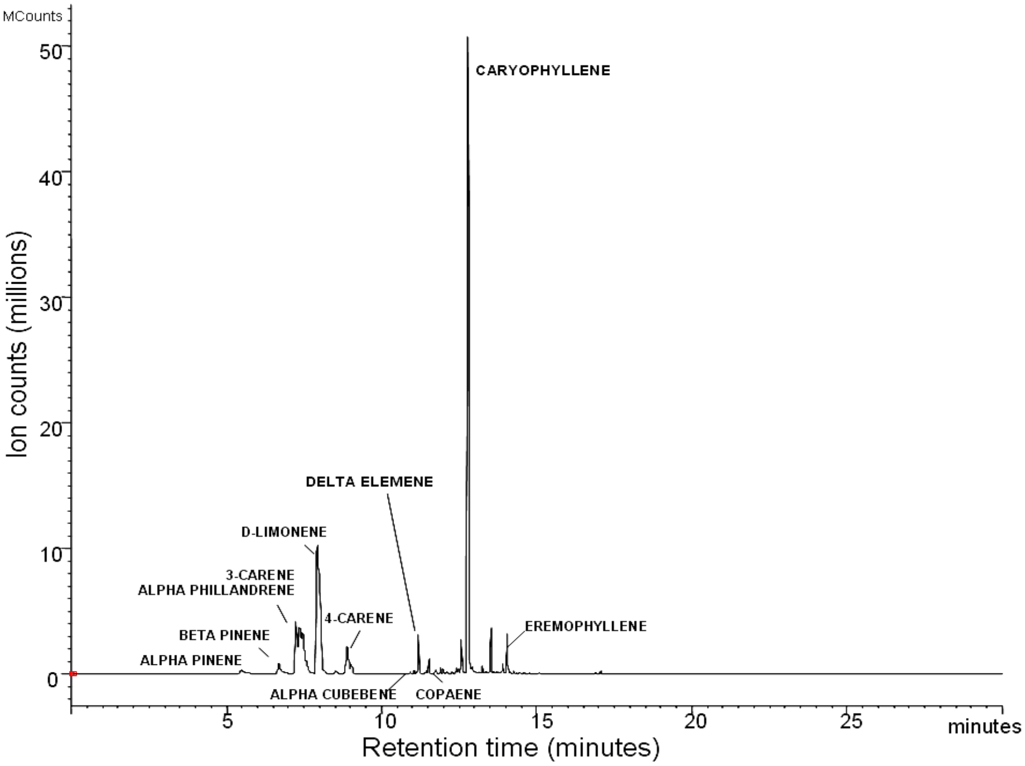


Figure 6.21 The GC-MS peaks of pepper showing major compounds.

The trend obtained for microwave field vs extraction for pepper is similar to what has been reported for higher power levels in pepper extractions. At higher Field strength (as also equivalent power levels), the confluence of smaller lipid bodies into larger agglomerates occurs swiftly, leading to a rapid cell rupture and an increased rate of extraction (Raman 2002).

ANOVA was performed to see whether a categorical independent variable (RMS Microwave field strength) has an effect on some continuous dependent variable (essential oil extraction) using SAS.

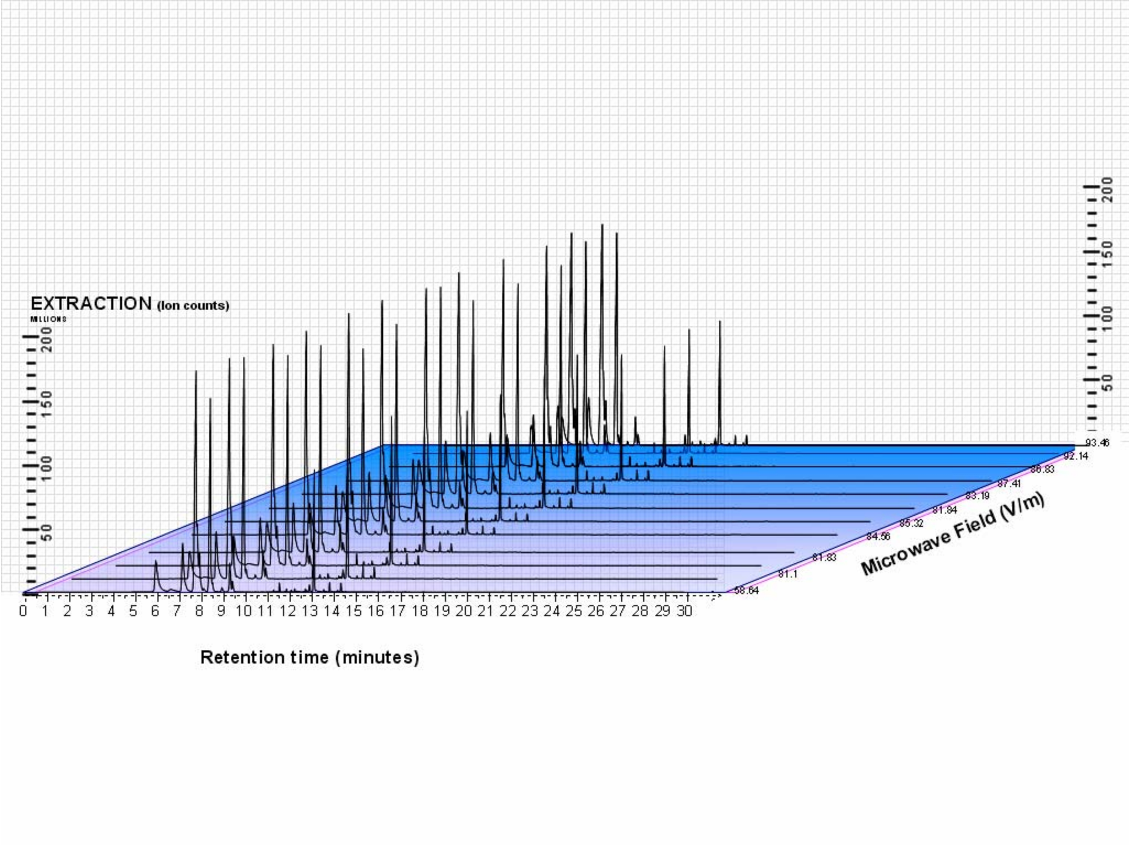


Figure 6.22 The GC-MS plots of extraction of black pepper at different fields.

One way ANOVA was performed with field as the independent variable or factor (An ANOVA with n factors is referred to as an n-way ANOVA). The analysis of variance was done on ten fields. The eleventh field at position L0 was not considered since sparking and erratic readings often showed up at L0 which also happens to be at close proximity to the wheels and belt. R^2 is an indicator of how well the current procedure fits the data. It is defined as the ratio of the sum of square for the model to the sum of square for the corrected total. Above, we have R^2 to be 0.456444 or 46% of the variability of the extraction can be explained by this statistical procedure. Pr answered the question, do the independent variables reliably predict the dependent variable. Here Pr of 8.1% showed a low chance of fluke and that indeed there was a trend present. Duncan grouping revealed similarity in the effect of treatment. The following fields showed similar effects on extraction as indicated by SAS letterings. The ANOVA result is given in Appendix G.

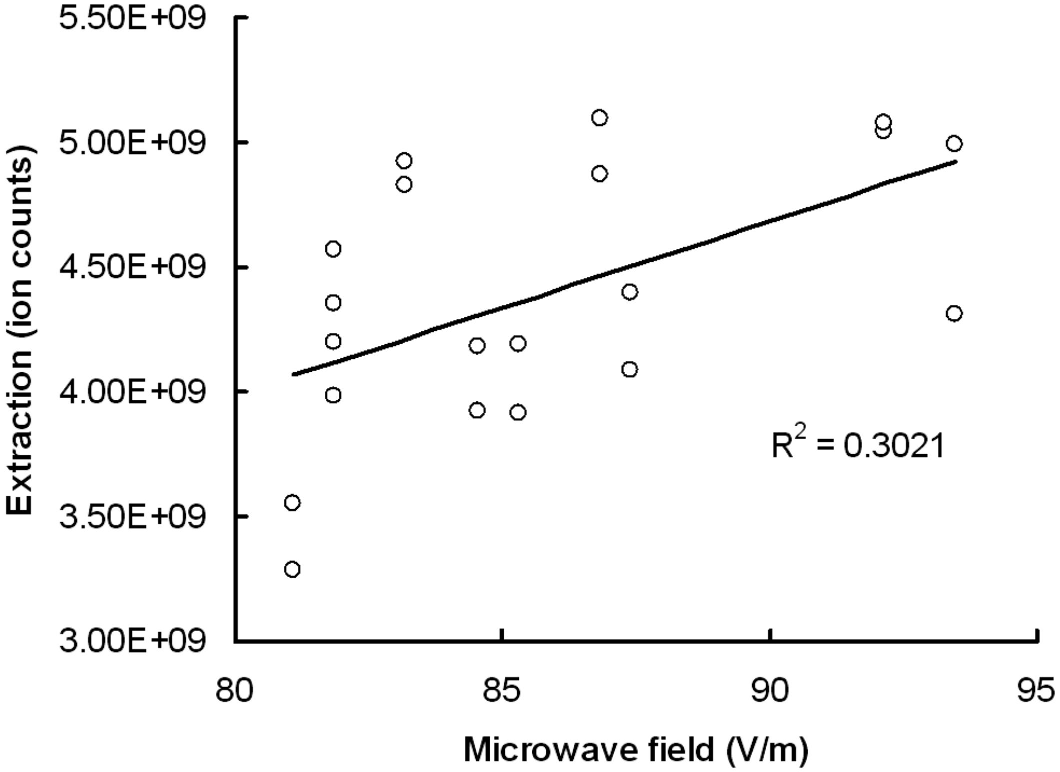


Figure 6.23 The effect of field variation on extraction of dry pepper powder.

6.4 Effect of Microwave Field Variation on Coriander Extraction.

The effect of field variation on coriander extraction was performed at five major fields since the GC-MS of the coriander indicated a poor response of coriander to microwave extraction. The GC-MS data obtained for coriander extractions are summarized in Table 6.11.

Table 6.10 The effect of microwave field on extraction of dry coriander.

Sl no	Expt Name	Location (mm)	Field (V/m)	Cumulative ion counts	Linalool ion counts
1	L1.1	198.95	81	3×10^7	1.12×10^6
2	L1.2	198.95	81	3×10^7	1.13×10^6
3	L1.3	198.95	81	3×10^7	1.11×10^6
4	ML.1	171.78	85	3×10^7	1.13×10^6
5	ML.2	171.78	85	3×10^7	1.13×10^6
6	ML.3	171.78	85	3×10^7	1.13×10^6
7	R5.1	80.62	92	3×10^7	1.10×10^6
8	R5.2	80.62	92	3×10^7	1.14×10^6
9	R5.3	80.62	92	2×10^7	1.11×10^6
10	R3.1	50.62	87	2×10^7	1.10×10^6
11	R3.2	50.62	87	3×10^7	1.10×10^6
12	R3.3	50.62	87	3×10^7	1.10×10^6
13	R2.1	35.62	87	2×10^7	1.09×10^6
14	R2.2	35.62	87	2×10^7	1.07×10^6
15	R2.3	35.62	87	2×10^7	9.02×10^5

The extraction trend for coriander were obtained from the GC-MS profiles shown in Figure 6.24 and data in Table 6.11. The trends were found to be marginally varying and showing minimal responsiveness to microwave extraction.

The ANOVA was performed for the above extractions of coriander in SAS. The R^2 value was 0.57936 showing the statistical procedure was reliable in explaining to the extent of 58% of the trend. The Duncan grouping showed 4 out of 5 microwave field factors had the same lettering or that 4 out of 5 fields had no significant effect on extraction. The statistical analysis indicated that the extraction of coriander was not responsive to field variation in the current experimental conditions. The ANOVA result for coriander is given in Appendix G.

Coriander is found to be less responsive in dry condition to microwave extraction. The rehydration of the coriander powder is likely to improve the extraction but it needs great care because of the significant percentage of high-boiling oils present in coriander (which makes it a potential edible oil crop).

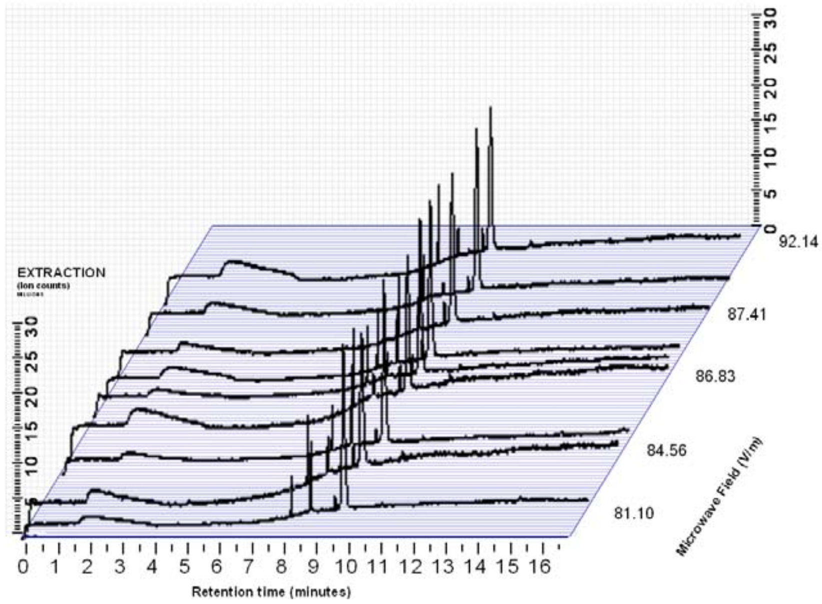


Figure 6.24 GC-MS plots for coriander extracts at different MW fields overlaid.

The oil present in coriander may hydrolyze in the presence of water content. But it is more important to note that the oil makes the moist coriander pasty, thereby adversely affecting the flow of the particulates. Flowability is important for even distribution and thin layer formation of particulates towards uniform microwave exposure. The water content could still be enhanced in the form of incorporated moist alumina particulates along with coriander powder as discussed in chapter 8.

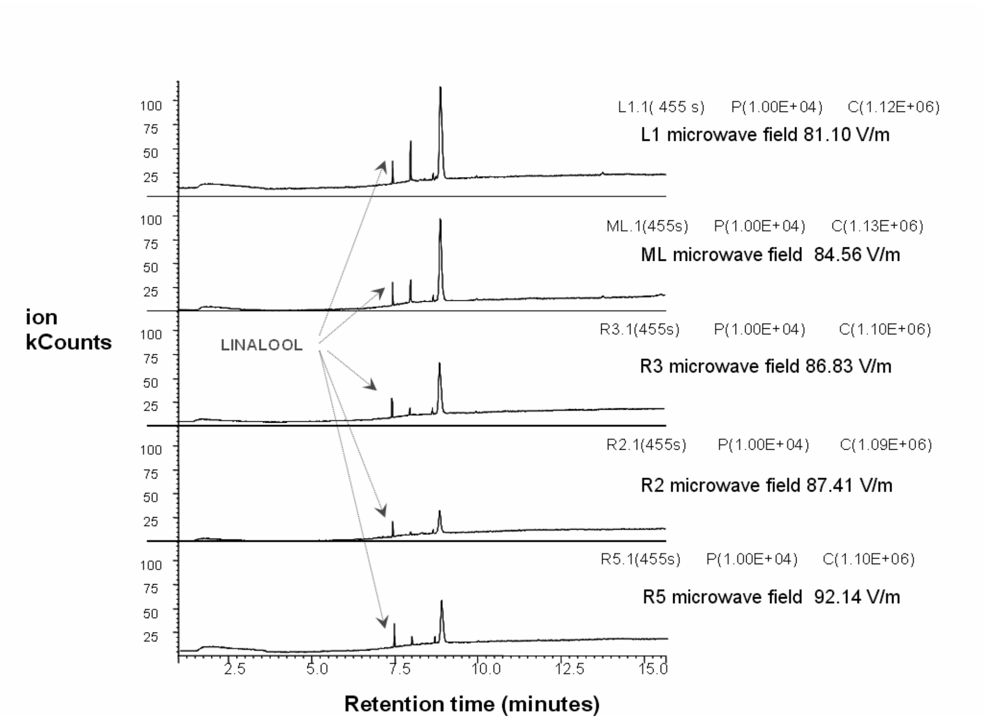


Figure 6.25 Stacked GC-MS plots for coriander extraction at different microwave fields.

GC-MS plots of the coriander extracts are shown overlaid in Figure 6.24 where as 6.25 shows the same plots stacked. In Figure 6.25, the linalool peaks are marked so as to highlight the lack of impact felt in coriander extraction by varying the microwave field.

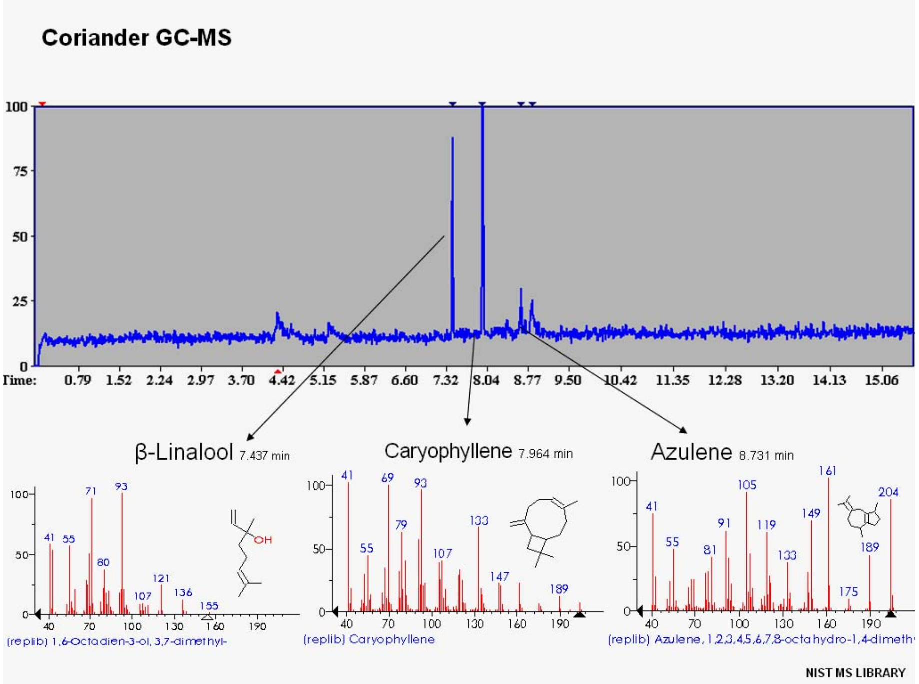


Figure 6.26 GC-MS peaks for coriander extracts showing major compounds.

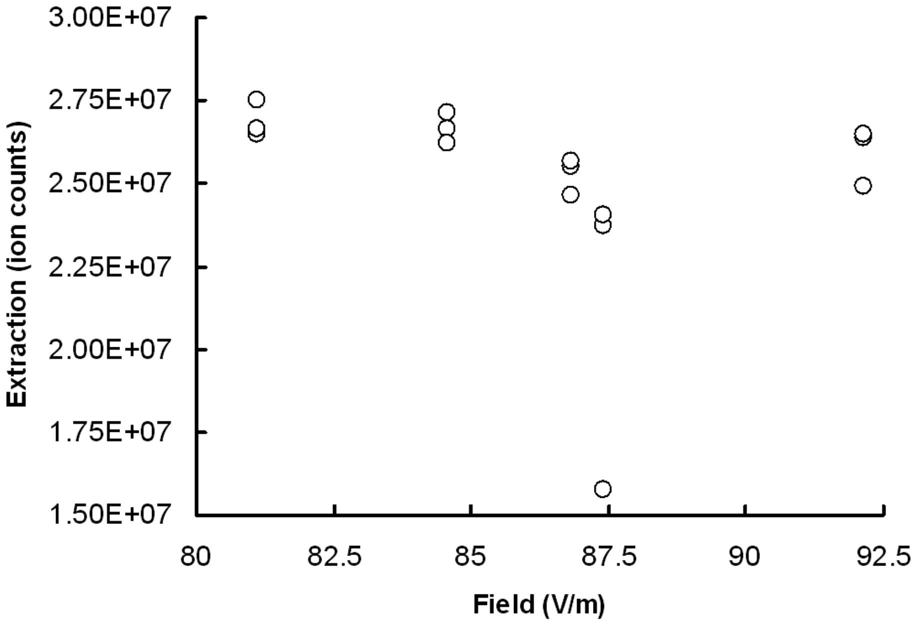


Figure 6.27 The effect of electric field variation on coriander extraction.

Figure 6.26 shows the retention time of linalool as 7.32 minutes. Figures 6.27 and 6.28 show the trends for coriander extraction in terms of field versus ion counts. Figure 6.27 gives the extraction in the units of cumulative ion counts and Figure 6.28 gives the extraction in the units of linalool ion counts. Linalool is the major component of the coriander extract and can be used to represent the extraction. It can be seen that the two trends 6.27 and 6.28 are identical to one another.

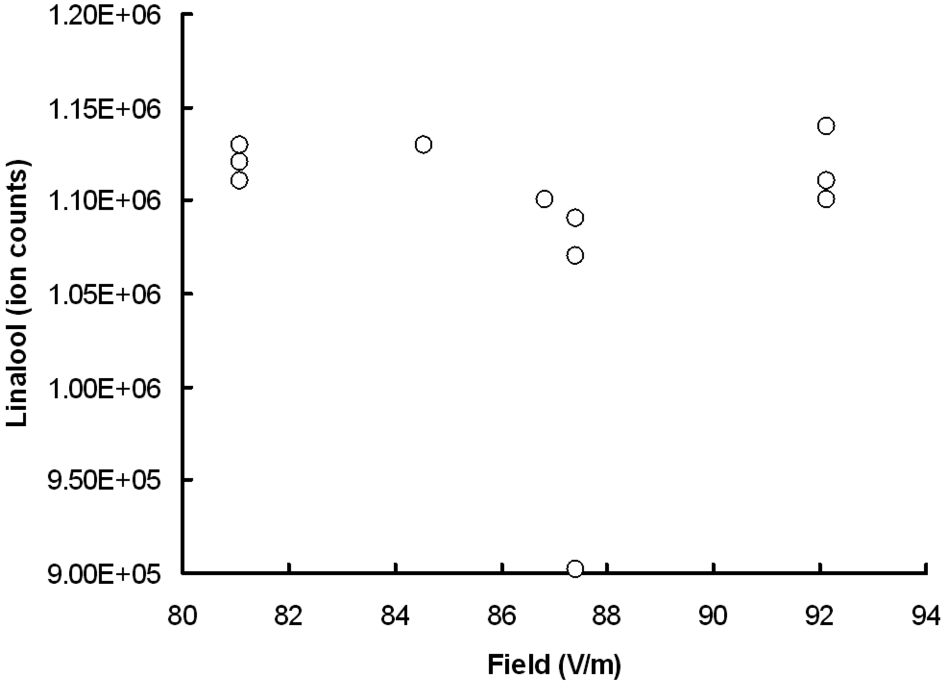


Figure 6.28 The effect of electric field variation on coriander extraction based on only Linalool GC-MS peak area.

6.5 Comparison between coriander and pepper extraction

The trend was found to be the same for linalool peak area comparison as well as cumulative peak area comparison for coriander. (Figures 6.27 and 6.28).

Comparatively, coriander shows only weak response to microwaves. Whereas pepper responds positively and linearly to field increase of microwaves. The reason for poor response of coriander may be due to the presence of significant amount of lipids (upto 22% by weight) as discussed briefly in section 6.4. For nonpolar compounds, such as lipids, only atomic polarizations (Ra) and electronic polarizations (Re) are predominant. Atomic polarizations arise from the relative displacement of the nuclei to the unequal charge distribution within the molecule, and electronic polarization arises from the realignment of the electrons around the specific nuclei. These polarizations do not relate to microwave absorption (Raman 2002). Hence there is no heating of the lipids. Moreover, the lipids also dissolve essential oils leaving insignificant amounts to be detected in the headspace. This could change at higher temperatures which can be brought about by water content increment. The enhancement of water content directly on coriander powder only makes it pasty and adversely affects flow, so it needs to be introduced on inert particulates such as alumina or other non-metallic particles.

6.6 Effect of Rehydration on Pepper Extraction.

The effect of increased water content on extraction of pepper was studied. The study was conducted to test the procedure towards obtaining expected results. Water content increase proportionately increases the microwave interaction with the biomaterial and with increased heating, the extraction is also expected to increase.

6.6.1 Effect of field variation on pepper extraction at 15% rehydration

For a constant water content the extraction was found to increase with an increase in microwave field strength as seen in Figure 6.29. The purpose of the rehydration experiments was to observe the effects that are significantly different due to the presence of additional water. The observation was that the presence of water enhances the particulate behavior of pepper powder owing to increased density of granules. The tumbling motion during extraction leads to better mixing and better thin layer spread when compared to dry state of the pepper powder.

Table 6.11 Effect of field variation on pepper extraction at 15 % water content.

SL	Expt	Location (mm)	Field (V/m)	MC (% wb)	Extraction
1	4W4MLb	171.78	85	15	1.57×10^{7}
2	4W4MLa	171.78	85	15	1.39×10^{7}
4	4W4R0a	15.62	82	15	1.36×10^{7}
5	4W4R0b	15.62	82	15	1.39×10^{7}
7	4W4L0a	198.95	59	15	1.23×10^{7}
8	4W4L0b	198.95	59	15	1.29×10^{7}
10	4W4MRa	95.62	85	15	1.28×10^{7}
11	4W4R5a	80.62	92	15	1.47×10^{7}

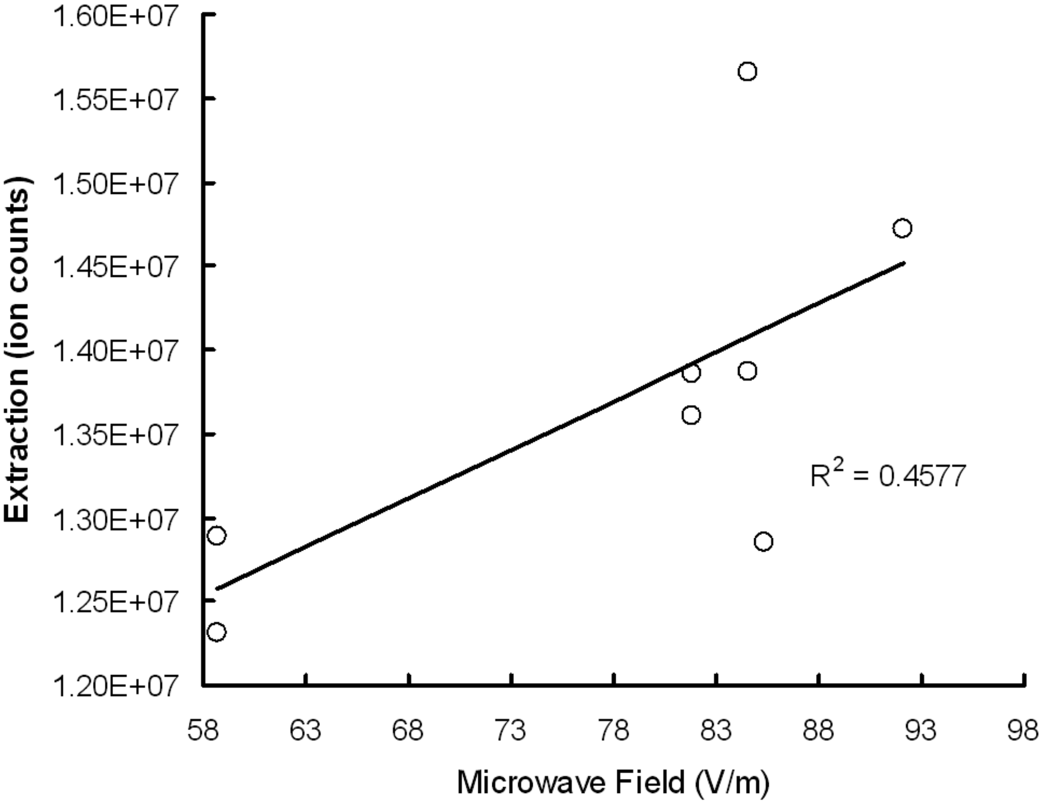


Figure 6.29 Effect of variation in electric fields on moist pepper extraction.

6.6.2 Effect of incremental rehydration on pepper extraction at a constant field

Based on the same argument as given in section 6.6.1, the water content increase keeping the microwave field strength constant, leads to a corresponding increase in extraction.

The trend shown by increased rehydration in three steps at a constant field position R5 are given in Table 6.12 and Figure 6.30. The experiment showed the expected enhancement in extraction owing to attainment of greater heating in the presence of high water content.

Table.6.12 Effect of added water on pepper extraction at constant field.

Sl No	Location	Location(mm)	Field (V/m)	Water content (x sample mass)	Extraction (ion counts)
1	WR50mL45	80.62	92	0	7.19×10^{7}
2	WR55mL45	80.62	92	1.25	1.56×10^{8}
3	WR510mL45	80.62	92	2.5	2.73×10^{8}

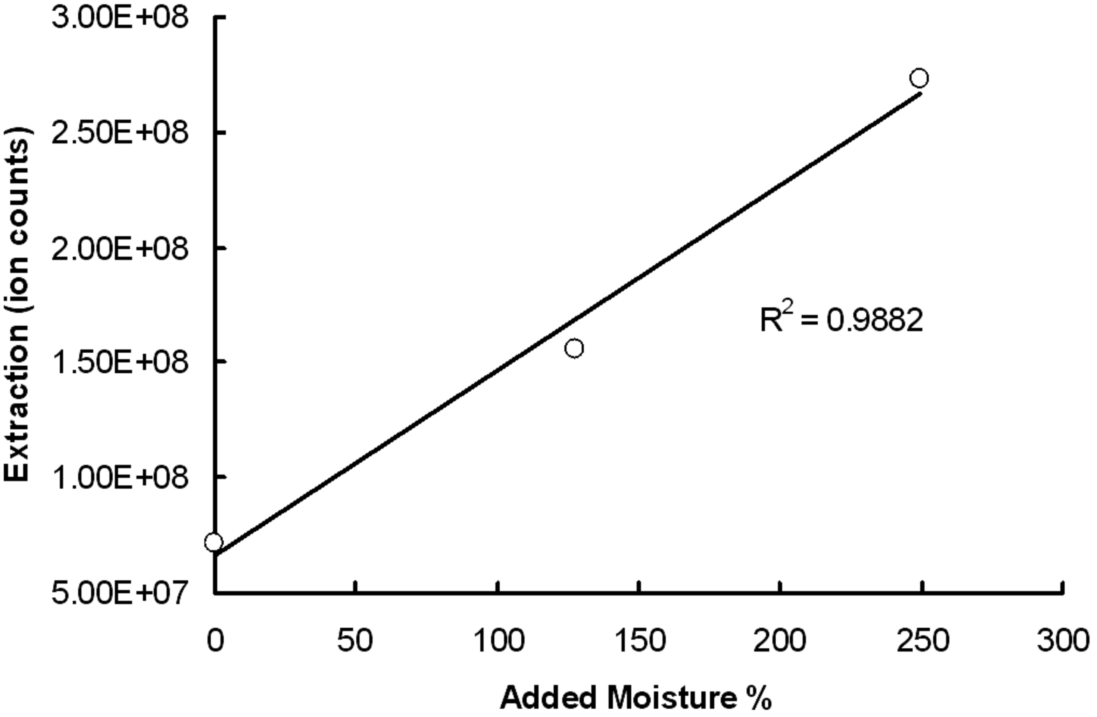


Figure 6.30 Effect of increasing water content on pepper extraction at constant field.

The trends seen in 6.6.1 and 6.6.2 indicate that the water content accenuates the trends seen in the case of dry extraction.The water content increment accentuates the trend seen in microwave field variation on extraction. At a constant field the extraction bears a direct relationship with water content increment It has been pointed out in the literature (Raman 2002) that the dielectric permittivity of biopolymers increases with the degree of hydration and, therefore, leads to a rapid dielectric heating. Water within the pepper cell forms a tightly bound primary monolayer, adsorbed on the protein and lipid molecules, causing a larger dielectric loss and thus increasing the rate of dielectric heating. Although the trend appeared to conform with the theory of microwave heating and intuitive reasoning. The ANOVA performed in SAS showed that the significance of the treatments were low for both the cases(6.6.1, 6.6.2) of low (15%) as well as high (up to three times mass of sample) water content increment. The Duncan grouping for the low water content treatment showed that all the field values belonged to the same group. The trends plotted, according to SAS can not be taken to indicate clearly as effects of the independent variable.

6.7 Effect of the Charecteristics of Substrate on Extraction

In order to understand the effect of solvent on the extraction, experiments were performed at constant microwave field position R5 with constant weight of pepper powder.

The various samples tested were as follows: Pepper corn before pulverization and powdered pepper with and without added water and addition of alcohol ethanol (100%) to powdered pepper. The trend obtained is given in Table 6.14 and the histogram of the trend is given in Figure 6.31.

Ethanol is a green solvent and may be a good solvent to use in microwave extractions since it couples with microwave field well and also has affinity towards non-polar compounds obtained in plant extracts.

Table.6.13 Effect of microwave extraction with variations in substrates.

Sl no	Expt name	Location (mm)	Expt Name	Field (V/m)	Extraction (ion counts)
1	R5	80.62	Pepper corn	92	6.87×10^{8}
2	R5	80.62	16% water content	92	7.87×10^{9}
3	R5	80.62	34% water content	92	8.38×10^{9}
4	R5	80.62	9.5% Ethanol	92	1.41×10^{9}
5	L0	198.95	0% moisture	59	3.45×10^{9}

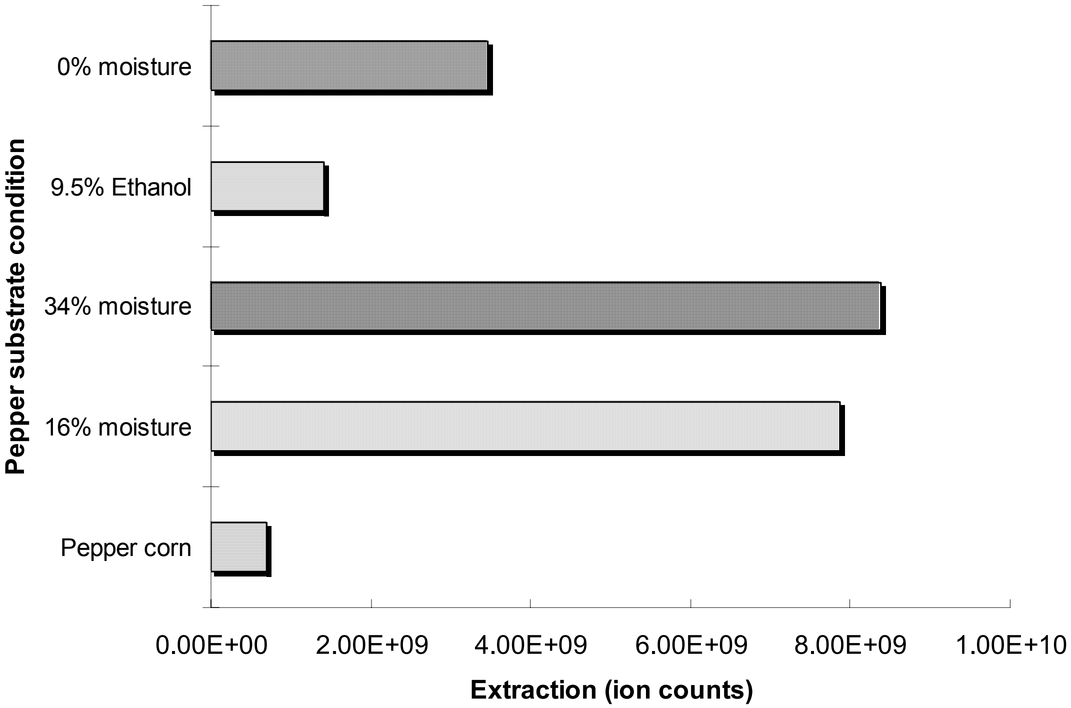


Figure 6.31 Effect of microwave extraction with variations in substrates.

It has been reported that small amounts of alcohol is likely to increase the extraction through dramatic increase in coupling (Mingos 1991). This was not observed in the test conducted. Ethanol has a dielectric loss value of 1.6 (Raman 2002) compared to 10 for water. Moreover it dissolves essential oil (like dissolves in like, ethanol has a non-polar CH^3CH^{2+} attached to OH^- where as water has H^+), hence the volatiles might have shown low concentration in the headspace. Hence extraction in the presence of ethanol is found to be less than that in its absence (indicated by 0% water content bar).

The water content showed a predicted trend of increasing the extraction with an increase in rehydration. The pepper corn shows very little response to microwaves. It however has a significant peak in the range of caryophyllene retention time (11 to 15 minutes) which might include elemene and eremophyllene.

7. SUMMARY AND CONCLUSIONS

Microwave energy is used in many industrial processes since it has the advantage of bringing about heating through kinetic effects inside the sample thereby improving efficiency and controllability of the process. By and large, microwave heating occurs through heating of water molecules present, even if in trace amounts, in the substrates. Biomaterials have a water content significant enough to be considered good substrates for microwave processing. Although each biomaterial responds differently to microwave exposure, they all heat faster and more uniformly in microwaves compared to other thermal treatments.

The main reason why this useful technology has not become as prevalant as conventional thermal technologies is partly due to the sensitive prerequisites for microwave processing. Microwaves may spark in the presence of metals and may incrementally heat a narrow region leading to detrimental hot spots depending on the interference patterns of electromagnetic waves in the heterogeneous absorbing media within the processing region.

It is possible to benefit from the microwave technology if the procedures are specifically designed for it. In the current research, modification of the existing laboratory microwave equipment (COBER™ electronics) was investigated by conceiving and designing a rotary vessel for extraction. The rotary extractor was found to give dependable results that were statistically analyzed. The rotary extractor is essential for uniform particulate distribution to prevent segregation based on density and size. In the course of its slow rotation, the extractor also distributes the particulates as a thin layer thereby ensuring total exposure of the sample to microwave field without any attenuation.

The current experiment successfully tested the procedure for following the microwave process in a sensitive extraction of spice volatiles (from black pepper and coriander). The findings of the current research may lead to development of industrial microwave extractors for flavors, fragrances and medicinal aroma.

New procedure for measuring microwave heating and sensing the extraction of volatiles from black pepper and coriander powder samples was developed. Heating could be measured consistently using an infrared thermometer and the extraction was sensed using solid phase micro extraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS).

The unique tumbling design for the extractor made it possible to measure the microwave field at the region of extraction and comparing extractions at different microwave fields. For a given homogeneous sample, the heating is proportional to microwave field that it is subjected to. Using water as the reference and measuring its temperature rise for a given microwave exposure time, it was possible to establish the microwave field profile inside the oven cavity at the region of extraction. In the COBER™ microwave oven used in the current research, the microwave field was maximum at the center and reduced at the extremes. The plot of microwave field versus position inside the oven cavity gave a dome shaped curve.

With the microwave field measured at the region of extraction, it was possible to plot pepper extraction versus the microwave field to which the pepper sample was exposed. The extraction was represented in terms of cumulative value of ion counts obtained in GC-MS analysis. This unique procedure developed in the current research allows for the graphical comparison of the microwave extractions. It was found that black pepper has a better response to microwave extraction than coriander. The pepper extraction was found to increase proportionately with increase in microwave field strength. The extraction was also enhanced proportionately by the incremental addition of water content at constant microwave field.

Coriander powder which has significant quantity of high-boiling lipids (between 12-20% of lipids in coriander do not volatilize below 100^0C , was found to respond poorly to microwave extraction in dry state. Increment of water content to coriander only lead to formation of a pasty state which prevented uniform distribution of particulates during extraction and formation of a thin layer, that was needed to ensure complete extraction.

The procedures developed for microwave extraction, SPME sampling and GC-MS analysis of the volatiles was found to yield results for black pepper that were in accordance with the present understanding of microwave extraction reported in the literature. The study of coriander is expected to require further understanding of water content inclusion or rehydration without affecting the flowability of the powder.

The current research has shown the feasibility of the monolithic procedure (Chapter 5, summarized in Figures 5.16 and 5.17) for extraction, sampling and analysis to follow microwave extraction. This methodology has been aptly named microwave mediated method (MMM), a new name to identify the unique systems engineered research described in this thesis.

8. RECOMMENDATIONS FOR FUTURE WORK

The research experiment opened new possibilities as well as new problems. In this section some of the ideas that occurred towards the end of the research, which on hindsight might have improved its results, are given.

In the rotational module the use of PVC and glass could be replaced by microwave transparent materials such as Teflon or frosted quartz. The use of steel screws also might be eliminated if ceramic or Teflon screws are available. This is because glass and PVC are marginal absorbers of microwave energy. Moreover, glass is reflective to the extent that it might indicate inaccurate temperature during infrared temperature measurement. The screws are spots where sparking as well as over heating might take place.

Sampling of volatiles is another area where it was felt that septum usage could be made more gas tight. One method is to discard a septum after use. In addition there can be fastening material used to tighten the grip of the septum.

Infrared temperature measurement used for microwave field measurement could be replaced with multi-pass fiber-optics sensor designed for rotational interface.

The extraction step has several improvisations that are possible. Mainly, it is inclusion of water content without extensively wetting the spice powder itself. This can be achieved by including water content in the form of wetted alumina (or other non-metallic) particulates.

The analysis of the GC-MS towards comparing the extraction could be done quantitatively by selectively spiking the powder to arrive at a reference curve with which the GC-MS plot of microwave extract could be juxtaposed. The spiking would have to be done extensively to give rise to sufficient learning of an artificial neural network algorithm so as to arrive at a level where useful patterns can be mined.

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APPENDIX

Table of contents for the Appendix

APPENDIX A

Secondary metabolism- a brief write-up.

Plants are exceptionally versatile chemical factories since their survival rests on this chemical response to environmental stress.

Many natural products were originally investigated for their medicinal, perfumery and culinary value, deriving inspiration from several aboriginal cultures that have sophisticated and predominantly plant dependent lifestyle.

The process of chemical synthesis taking place in living cells is called metabolism. There are three kinds of metabolic products. The ***primary metabolites*** such as the C18 oils, starch, sugars etc. form the basis of agriculture. High molecular weight polymers such as lignin, cellulose etc, the ***structural metabolites***, mostly constitute the forest products (viz. timber, paper etc.) Then there are those small volume plant extracts that have attracted a commercially strong niche for themselves, due to their high value preservative, medicinal, fragrance and flavor characteristics. These are the ***Secondary metabolites***- the subject of this work (Figure A.1).

As the names suggest, primary metabolites are essential for sustenance; the structural metabolites define membranes and maintain the plant structure and the secondary metabolites are sort of elbowroom for plant's evolution!

Secondary metabolites have varied biological activity. They play an important role in regulating the interaction between plants, micro-organisms, animals and insects. The aroma of spices, fragrance of flowers, tinctures of eucalyptus, lavender and basil are examples of secondary metabolites.

Although the chemical compounds from secondary metabolism are phenomenally varied, organic chemists have strived to classify them in essentially six categories based on biosynthetic pathways (Dewick 2002). These are 1.)acetate pathway; 2.)Shikimate pathway;3.) Mevalonate and Deoxyxylulose phosphate pathway; 4.) Alkaloids, Peptides, proteins etc and 5.)Carbohydrates. Most predominant among them are *Terpenoids* - the cause of aroma. Terpenoids come from mevalonate and deoxyxylulose phosphate pathways. The terpenoid, is of primary interest to us here. Most important natural aroma are associated with members of this class. Terpenoids are the most structurally varied class of natural products. The name comes from the fact that earliest compounds of this type were isolated from turpentine. Terpenes are aromatic (smelly) compounds. They are formed from isoprene units. They have a cyclic structure resulting from acid-catalyzed rearrangements (made possible by their branched chain and easily protonated unsaturation sites)

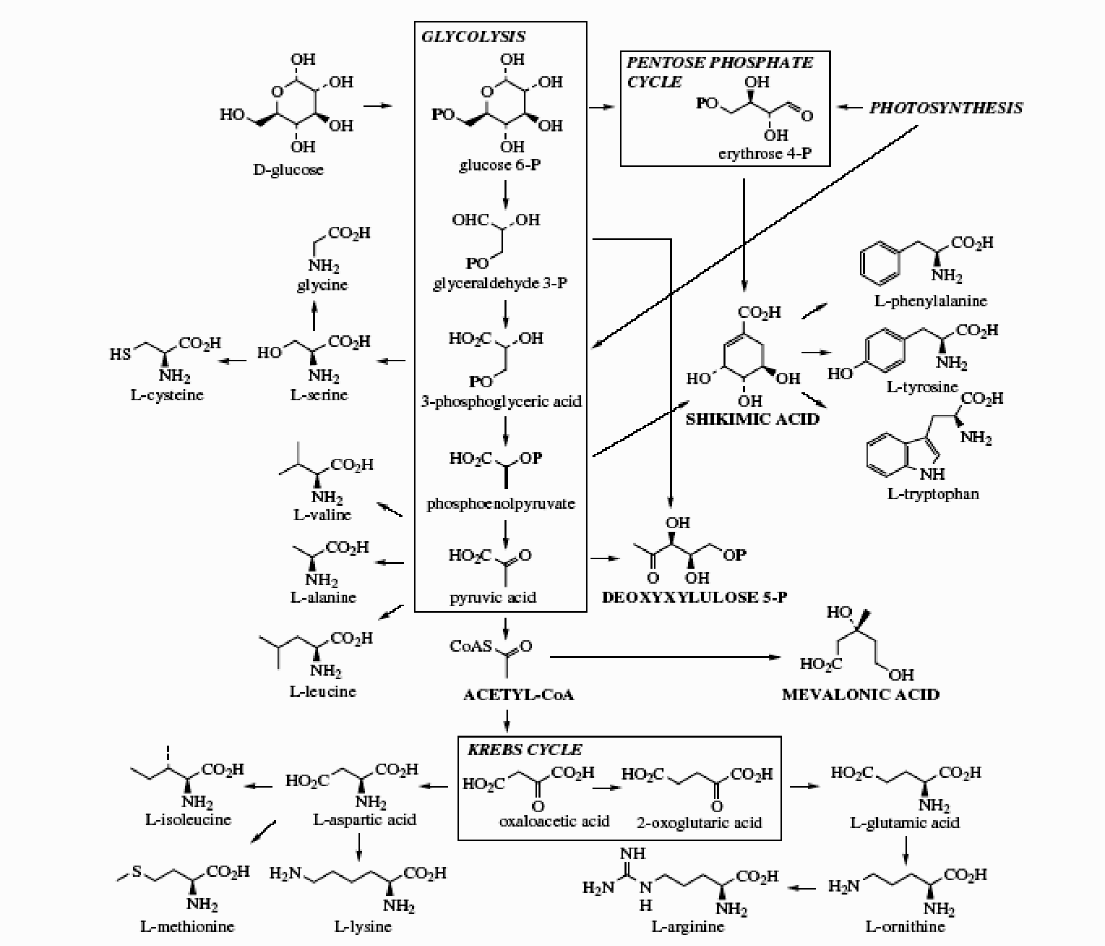


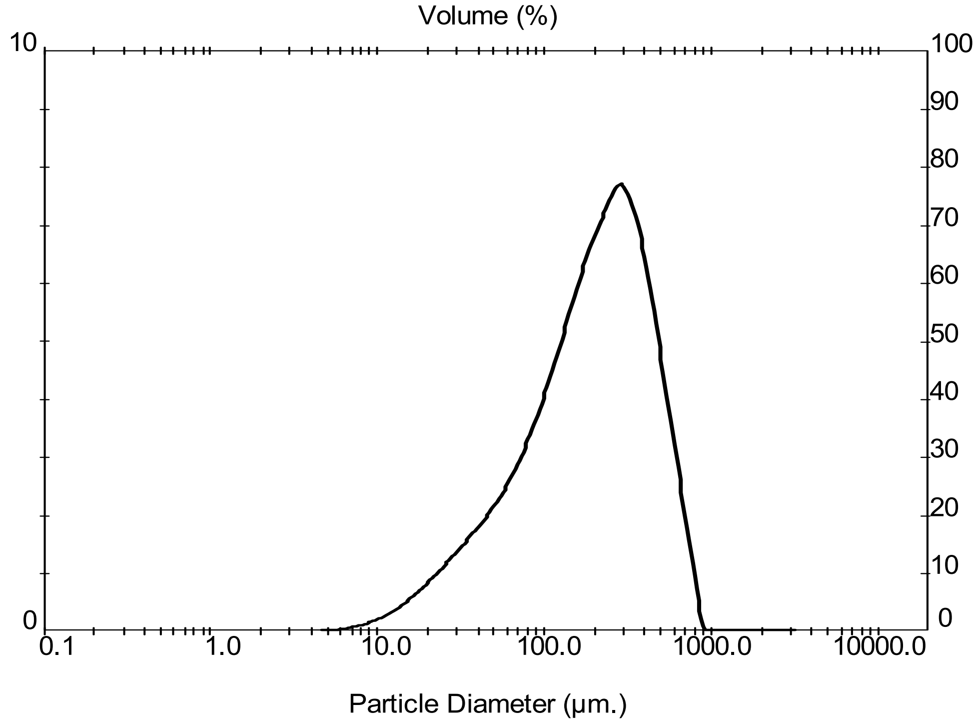
Figure.A.1 The biosynthesis of secondary metabolites (Dewick 2002)

Monoterpenoids are major components of the aroma in plants. They have been extensively studied for their spicy, citrus and various other exotic flavors and fragrances. These volatile natural products, known as essential oils form the basis of the perfumery and flavoring industries. The structures of many of the simple monoterpenes were established between 1890 and 1920 through the work of Wallach, Wagner, Tiemann, Semmler and Perkin.

Monoterpenes are volatiles with characteristic herbal odor. Almost all spices derive their fragrance from monoterpenes.

APPENDIX B

B.1 Particle size analysis - Coriander



Result: Analysis Table

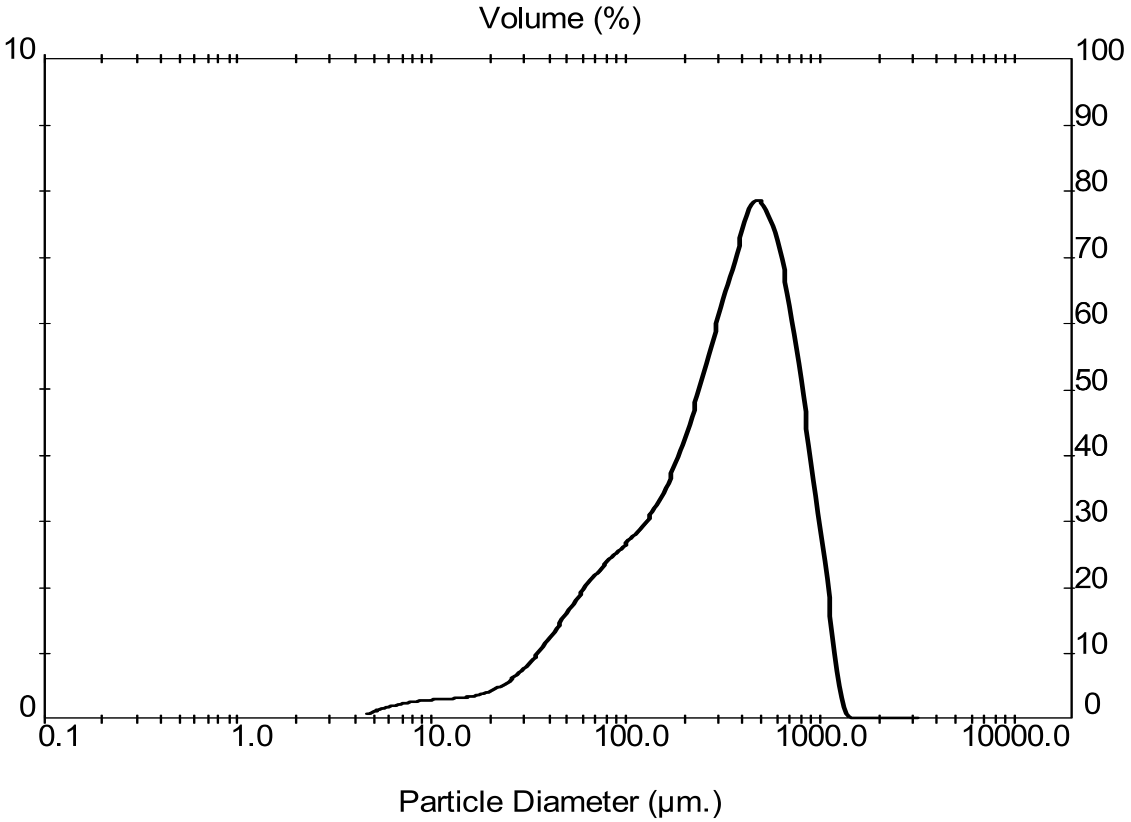
ID: coriander　Run No: 5　Measured: 15/11/2004 14:16PM
File: BALA　Rec. No: 6　Analysed: 15/11/2004 14:16PM
Path: C:\SIZERS\DATA\　Source: Analysed

Range: 1000 mm　Beam: 10.00 mm　Sampler: MS64　Obs': 0.8 %
Presentation: 3PAD　Analysis: Polydisperse　Residual: 0.515 %
Modifications: None

Conc. = 0.0028 %Vol　Density = 1.000 g/cm^3　S.S.A.= 0.0592 m^2/g
Distribution: Volume　D[4, 3] = 237.05 um　D[3, 2] = 101.39 um
D(v, 0.1) = 45.04 um　D(v, 0.5) = 200.84 um　D(v, 0.9) = 483.46 um
Span = 2.183E+00　Uniformity = 6.741E-01

Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %
4.19	0.01	22.49	1.09	120.67	5.13	647.41	1.99
4.88	0.03	26.20	1.30	140.58	5.77	754.23	0.75
5.69	0.05	30.53	1.51	163.77	6.38	878.67	0.00
6.63	0.08	35.56	1.73	190.80	6.90	1023.66	0.00
7.72	0.13	41.43	1.97	222.28	7.33	1192.56	0.00
9.00	0.20	48.27	2.24	258.95	7.69	1389.33	0.00
10.48	0.29	56.23	2.55	301.68	7.43	1618.57	0.00
12.21	0.40	65.51	2.94	351.46	6.75	1885.64	0.00
14.22	0.54	76.32	3.39	409.45	5.71	2196.77	0.00
16.57	0.71	88.91	3.91	477.01	4.47	2559.23	0.00
19.31	0.89	103.58	4.50	555.71	3.23	2981.51	0.00
22.49		120.67		647.41		3473.45	

B.2 Particle size analysis - Pepper



Result: Analysis Table

ID: pepper Run No: 8 Measured: 15/11/2004 14:46PM
File: BALA Rec. No: 10 Analysed: 15/11/2004 14:46PM
Path: C:\SIZERS\DATA\ Source: Analysed

Range: 1000 mm Beam: 10.00 mm Sampler: MS64 Obs': 0.8 %
Presentation: 3PAD Analysis: Polydisperse Residual: 0.715 %
Modifications: None

Conc. = 0.0031 %Vol Density = 1.000 g/cm^3 S.S.A.= 0.0508 m^2/g
Distribution: Volume D[4, 3] = 372.39 um D[3, 2] = 118.00 um
D(v, 0.1) = 58.30 um D(v, 0.5) = 325.15 um D(v, 0.9) = 764.01 um
Span = 2.170E+00 Uniformity = 6.761E-01

Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %
4.19	0.09	22.49	0.56	120.67	3.04	647.41	6.24
4.88	0.15	26.20	0.71	140.58	3.37	754.23	4.90
5.69	0.20	30.53	0.91	163.77	3.80	878.67	3.38
6.63	0.25	35.56	1.15	190.80	4.36	1023.66	1.85
7.72	0.28	41.43	1.42	222.28	5.02	1192.56	0.33
9.00	0.30	48.27	1.71	258.95	5.74	1389.33	0.00
10.48	0.31	56.23	1.98	301.68	6.46	1618.57	0.00
12.21	0.32	65.51	2.22	351.46	7.15	1885.64	0.00
14.22	0.35	76.32	2.43	409.45	7.78	2196.77	0.00
16.57	0.39	88.91	2.61	477.01	7.75	2559.23	0.00
19.31	0.46	103.58	2.81	555.71	7.23	2981.51	0.00
22.49		120.67		647.41		3473.45	

APPENDIX C

C.1 Volume and height of a partially filled horizontal ampule

Volume = $L \times [(r^2 \mathrm{acos}((r-h)/r) - ((r-h) \times (2rh - h^2)^{1/2}$

L	Length of the cylinder	**h**	depth of water in horizontal position
r	radius of the cylinder	**acos**	$\cos^{-1}$

L(cm)	r(cm)	h(cm)	V(cm)	L(cm)	r(cm)	h(cm)	V(cm)
11.091	1.328	0.01	0.024073	11.091	1.328	0.52	8.486164
11.091	1.328	0.02	0.068012	11.091	1.328	0.57	9.675632
11.091	1.328	0.03	0.124804	11.091	1.328	0.62	10.90368
11.091	1.328	0.04	0.191929	11.091	1.328	0.67	12.1667
11.091	1.328	0.05	0.267923	11.091	1.328	0.72	13.46137
11.091	1.328	0.06	0.351791	11.091	1.328	0.77	14.78461
11.091	1.328	0.07	0.442799	11.091	1.328	0.82	16.13354
11.091	1.328	0.08	0.540374	11.091	1.328	0.87	17.50545
11.091	1.328	0.09	0.644054	11.091	1.328	0.92	18.89774
11.091	1.328	0.1	0.753453	11.091	1.328	0.97	20.30794
11.091	1.328	0.11	0.868244	11.091	1.328	1.02	21.73367
11.091	1.328	0.12	0.988142	11.091	1.328	1.07	23.17263
11.091	1.328	0.13	1.112901	11.091	1.328	1.12	24.62258
11.091	1.328	0.14	1.242299	11.091	1.328	1.17	26.08132
11.091	1.328	0.15	1.376138	11.091	1.328	1.22	27.54671
11.091	1.328	0.16	1.514241	11.091	1.328	1.27	29.01663
11.091	1.328	0.17	1.656444	11.091	1.328	1.32	30.48897
11.091	1.328	0.18	1.8026	11.091	1.328	1.37	31.96165
11.091	1.328	0.19	1.952571	11.091	1.328	1.42	33.43257
11.091	1.328	0.2	2.106231	11.091	1.328	1.47	34.89964
11.091	1.328	0.21	2.263461	11.091	1.328	1.52	36.36075
11.091	1.328	0.22	2.424153	11.091	1.328	1.57	37.81374
11.091	1.328	0.23	2.588202	11.091	1.328	1.62	39.25646
11.091	1.328	0.24	2.755512	11.091	1.328	1.67	40.68667
11.091	1.328	0.25	2.925992	11.091	1.328	1.72	42.1021
11.091	1.328	0.26	3.099555	11.091	1.328	1.77	43.50038
11.091	1.328	0.27	3.276121	11.091	1.328	1.82	44.87909
11.091	1.328	0.28	3.45561	11.091	1.328	1.87	46.23566
11.091	1.328	0.29	3.63795	11.091	1.328	1.92	47.56743
11.091	1.328	0.3	3.82307	11.091	1.328	1.97	48.87157
11.091	1.328	0.31	4.010903	11.091	1.328	2.02	50.14507
11.091	1.328	0.32	4.201383	11.091	1.328	2.07	51.38468
11.091	1.328	0.33	4.39445	11.091	1.328	2.12	52.58691
11.091	1.328	0.34	4.590044	11.091	1.328	2.17	53.7479
11.091	1.328	0.35	4.788108	11.091	1.328	2.22	54.86337
11.091	1.328	0.36	4.988587	11.091	1.328	2.27	55.92851
11.091	1.328	0.37	5.191427	11.091	1.328	2.32	56.93776
11.091	1.328	0.38	5.396579	11.091	1.328	2.37	57.88459
11.091	1.328	0.39	5.603992	11.091	1.328	2.42	58.76107
11.091	1.328	0.4	5.813618	11.091	1.328	2.47	59.55714
11.091	1.328	0.41	6.025411	11.091	1.328	2.52	60.25927

APPENDIX D

D The plots of Dielectric property of water

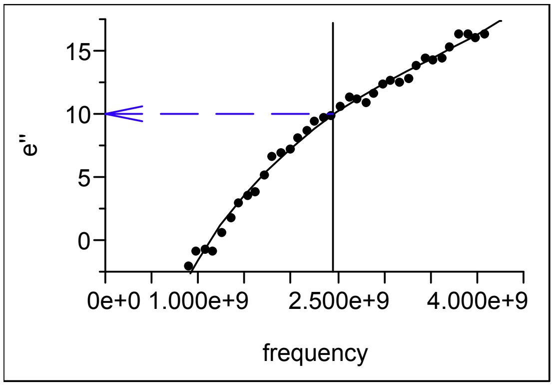


Figure D1 Dielectric loss Vs frequency

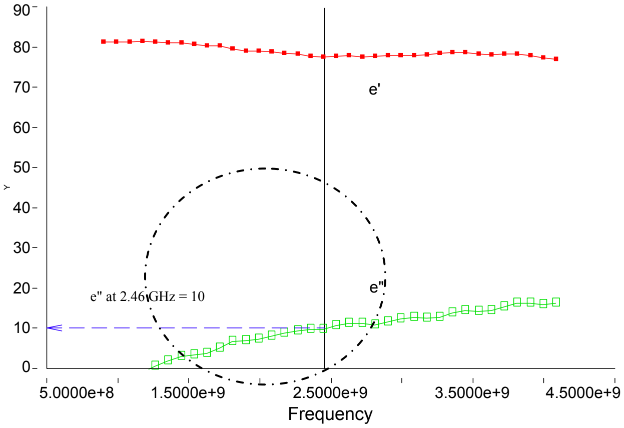


Figure D.2. Relative permittivity: real (red /upper) and imaginary (green/lower) part Vs frequency.

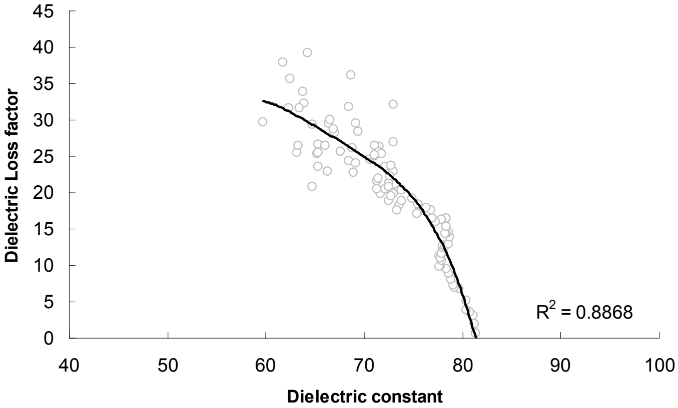


Figure D.3. Cole-Cole plot showing permittivity real Vs imaginary part.

APPENDIX E

Penetration depth Vs frequency for water

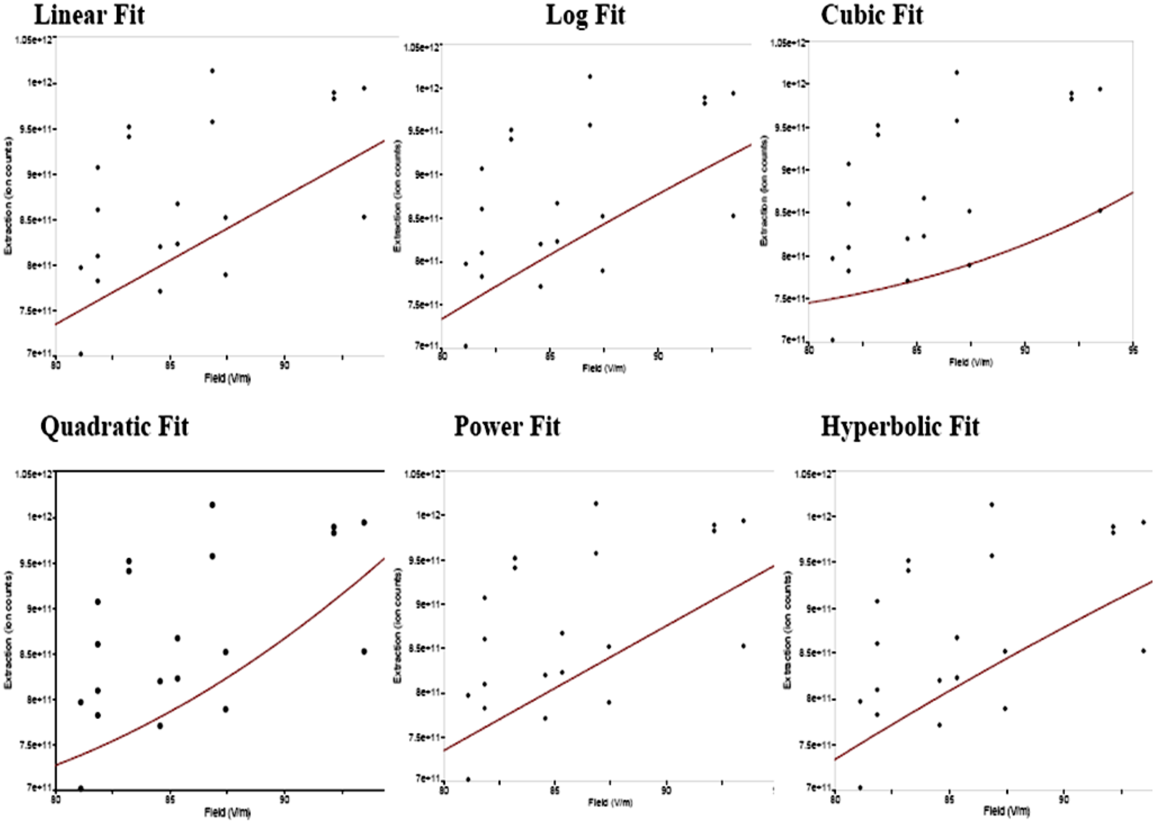
f(Hz)	**e'**	**e"**	**$e'^{1/2}$**	**l**(m)	**Dp=(l/2p)*($e'^{1/2}$/e")** (m)
1.26E+09	81.3211	0.6405	9.017821	0.237178	0.531466933
1.36E+09	81.1858	1.8952	9.010316	0.221249	0.167411978
1.45E+09	81.162	3.0733	9.008996	0.207325	0.096726162
1.54E+09	80.7206	3.5406	8.984464	0.19505	0.07877389
1.63E+09	80.3966	3.9112	8.966415	0.184148	0.067188547
1.72E+09	80.3623	5.1737	8.964502	0.174399	0.048093889
1.81E+09	79.6554	6.7274	8.924987	0.165631	0.034972173
1.9E+09	79.1523	7.015	8.896758	0.157703	0.031831918
1.99E+09	79.1357	7.3339	8.895825	0.150498	0.029053786
2.08E+09	78.8835	8.1379	8.881638	0.143923	0.024999556
2.17E+09	78.5627	8.8077	8.86356	0.137899	0.022086504
2.27E+09	78.3382	9.4868	8.850887	0.132359	0.01965349
2.36E+09	77.8225	9.7767	8.821706	0.127246	0.018273673
2.45E+09	77.6738	9.8921	8.813274	0.122514	0.017372233
2.54E+09	77.859	10.7152	8.823775	0.118122	0.015481155
2.63E+09	77.9376	11.3844	8.828227	0.114033	0.014073873
2.72E+09	77.6373	11.2311	8.811203	0.110218	0.013762104
2.81E+09	77.7135	10.9299	8.815526	0.10665	0.013690271
2.9E+09	77.9136	11.6269	8.826868	0.103305	0.012482056
2.99E+09	78.0175	12.498	8.832752	0.100165	0.011266515
3.08E+09	78.0434	12.7309	8.834218	0.097209	0.010735826
3.18E+09	78.2326	12.5225	8.844919	0.094423	0.01061451
3.27E+09	78.5626	12.8573	8.863555	0.091792	0.010071237
3.36E+09	78.773	13.8898	8.875415	0.089304	0.009082015
3.45E+09	78.64	14.4362	8.86792	0.086947	0.00850046
3.54E+09	78.3454	14.295	8.851294	0.084711	0.008348008
3.63E+09	78.1963	14.4344	8.842867	0.082587	0.00805246
3.72E+09	78.3242	15.3871	8.850096	0.080568	0.007375176
3.81E+09	78.3999	16.4064	8.854372	0.078644	0.00675511
3.9E+09	77.942	16.3467	8.828477	0.076811	0.006602341
3.99E+09	77.346	16.0308	8.794657	0.075061	0.00655385
4.09E+09	76.9602	16.4139	8.772696	0.073389	0.006242666

f	**e'**	**e"**	**e'$^{1/2}$**	**l**	**Dp=(l/2p)*(e'$^{1/2}$/e")**
(Hz)				(m)	(m)
5.72E+09	72.9785	22.8659	8.542745	0.052384	0.003114772
5.81E+09	72.1174	23.5269	8.492196	0.051564	0.002962246
6E+09	71.3684	20.491	8.447982	0.049999	0.003280719
6.09E+09	72.7195	23.6727	8.527573	0.049251	0.002823678
6.18E+09	71.565	26.289	8.45961	0.048526	0.002485245
6.27E+09	70.6504	24.5925	8.405379	0.047821	0.00260134
6.36E+09	71.419	21.939	8.450976	0.047137	0.00288984
6.45E+09	72.6846	22.32	8.525526	0.046472	0.002825145
6.54E+09	71.4682	25.6242	8.453887	0.045826	0.002406226
6.63E+09	68.864	26.053	8.298434	0.045197	0.002291233
6.72E+09	68.4806	24.3322	8.275301	0.044585	0.002413323
6.82E+09	71.0284	25.0166	8.427835	0.04399	0.002358646
6.91E+09	72.9425	26.9079	8.540638	0.04341	0.00219293
7E+09	71.8168	25.3437	8.474479	0.042846	0.002280195
7.09E+09	68.979	22.7324	8.30536	0.042296	0.002459406
7.18E+09	66.329	22.9733	8.144262	0.04176	0.002356162
7.27E+09	65.3309	26.6283	8.082753	0.041237	0.001992151
7.36E+09	66.3876	29.5802	8.147859	0.040727	0.001785445
7.45E+09	69.3718	28.4671	8.328974	0.04023	0.001873338
7.54E+09	71.0788	25.1784	8.430824	0.039744	0.002118062
7.63E+09	69.1782	24.0734	8.317343	0.039271	0.002159414
7.73E+09	65.2538	25.298	8.077982	0.038808	0.001972237
7.82E+09	63.1383	25.4319	7.945961	0.038356	0.001907322
7.91E+09	65.3571	25.4589	8.084374	0.037915	0.001916178
8E+09	69.1381	29.5226	8.314932	0.037483	0.00168021
8.09E+09	68.5045	31.8038	8.276745	0.037062	0.001535064
8.18E+09	66.0147	26.4754	8.124943	0.036649	0.001790051
8.27E+09	64.7885	20.7637	8.04913	0.036246	0.002236284
8.36E+09	65.3074	23.4744	8.081299	0.035852	0.001964343
8.45E+09	63.9367	32.2748	7.996043	0.035466	0.001398433
8.54E+09	62.4159	35.6053	7.900373	0.035088	0.001239118
8.64E+09	67.0527	28.1997	8.188571	0.034718	0.001604509
8.73E+09	72.4645	20.8248	8.512608	0.034356	0.002235154
8.82E+09	71.0296	26.5	8.427906	0.034002	0.001721051
8.91E+09	62.3643	31.5349	7.897107	0.033654	0.001341335
9E+09	59.7147	29.6833	7.727529	0.033314	0.001380305
9.09E+09	66.9262	28.6674	8.180843	0.03298	0.001497913
9.18E+09	72.9997	32.1178	8.543986	0.032654	0.001382501
9.27E+09	68.7371	36.0551	8.290784	0.032333	0.001183304
9.36E+09	63.4126	31.55	7.963203	0.032019	0.001286217
9.45E+09	63.2659	26.457	7.953986	0.031711	0.001517293
9.55E+09	66.5788	30.058	8.159583	0.031408	0.001356978

APPENDIX F

F.1 Comparative plots for Pepper extraction under dry condition at different fields

Comparison of various trend fits for the scatter points of Extraction (y-axis) Vs microwave field (x-axis) plot



Linear Fit

Log Fit

Cubic Fit

Quadratic Fit

Power Fit

Hyperbolic Fit

Figure F.1 Effect of microwave field variation on black Pepper extraction. Comparison of the various trend fits after baseline processing and least-squares minimizations.

Baseline	DOF	SSE	r^2	DF Adj r^2	FitStdErr	Fstat
Constant	3	4.4599e+22				
Linear	2	1.449e+22	0.67509823	0.02529470	8.5119e+10	4.15570674
Quadratic	1	1.4904e+22	0.66583174	0.00000000	1.2208e+11	0.99625222
Cubic	1	1.449e+22	0.67509823	0.02529470	1.2038e+11	0.69261779
Logarithmic	2	1.449e+22	0.67509823	0.02529470	8.5119e+10	4.15570674
Exponential	1	4.4599e+22	1e-08	0.00000000	2.1119e+11	1e-08
Power	1	1.449e+22	0.67509823	0.02529470	1.2038e+11	2.07785337
Hyperbolic	1	1.449e+22	0.67509823	0.02529470	1.2038e+11	2.07785337

Baseline	a	b	c	d
Constant	8.371e+11			
Linear	-3.882e+11	1.4039e+10		
Quadratic	4.2911e+09	5.0484e+09	5.1214e+07	
Cubic	-9.833e+11	1.4361e+10	2.2969e+08	-1.766e+06
Logarithmic	-4.627e+12	1.2233e+12		
Exponential	9.9451e+11	-2.916e+11	6.18000000	
Power	-4.722e+11	2.0001e+10	0.93566584	
Hyperbolic	2.6784e+12	-5.492e+12	43.8776151	

SAS R0 (REFER 6.2.2)

```
                                   19:38 Wednesday, July 6, 2005   1

                    The ANOVA Procedure

                   Class Level Information

             Class          Levels    Values

             Duration_s_         5    23 63 123 203 283


                  Number of observations    13
                                   19:38 Wednesday, July 6, 2005   2

                    The ANOVA Procedure

Dependent Variable: Extraction__ion_counts_   Extraction (ion counts)

                                Sum of
 Source                 DF        Squares     Mean Square    F Value    Pr > F

 Model                   4    3.9862217E13    9.9655541E12      0.36    0.8322

 Error                   8    2.2300306E14    2.7875383E13

 Corrected Total        12    2.6286528E14


      R-Square     Coeff Var      Root MSE    Extraction__ion_counts_ Mean

      0.151645      21.99599       5279714                        24003077


 Source                 DF        Anova SS     Mean Square    F Value    Pr > F

 Duration_s_             4    3.9862217E13    9.9655541E12      0.36    0.8322
```

SAS L0 (REFER 6.2.3)

```
                                   19:38 Wednesday, July 6, 2005   1

                         The ANOVA Procedure

                        Class Level Information

                  Class          Levels    Values

                  Duration_s_          5    23 63 123 203 283


                       Number of observations    10
                                   19:38 Wednesday, July 6, 2005   2

                         The ANOVA Procedure

  Dependent Variable: Extraction__ion_counts_   Extraction (ion counts)

                                Sum of
     Source                 DF        Squares    Mean Square    F Value    Pr > F

     Model                   4    2.3298433E15    5.8246084E14      0.62    0.6700

     Error                  5    4.721951E15    9.443902E14

     Corrected Total           9    7.0517944E15


         R-Square     Coeff Var     Root MSE    Extraction__ion_counts_ Mean

         0.330390      78.66358      30730932                     39066278


     Source                 DF        Anova SS     Mean Square    F Value    Pr > F

     Duration_s_              4    2.3298433E15    5.8246084E14      0.62    0.6700
                                   19:38 Wednesday, July 6, 2005   3

                         The ANOVA Procedure

                   t Tests (LSD) for Extraction__ion_counts_

   NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                             rate.


                     Alpha                     0.05
                     Error Degrees of Freedom        5
                     Error Mean Square        9.444E14
                     Critical Value of t       2.57058


              Comparisons significant at the 0.05 level are indicated by ***.


                      Difference
                Duration_s_        Between
                Comparison          Means    95% Confidence Limits

                123  - 63        3577498    -75418879   82573875
                123  - 23       29733242    -42380254  101846738
                123  - 283       32417500    -64332907  129167907
                123  - 203       36620000    -42376377  115616377
                63   - 123      -3577498    -82573875   75418879
                63   - 23      26155744    -45957752   98269240
                63   - 283      28840002    -67910405  125590409
                63   - 203      33042502    -45953875  112038879
                23   - 123     -29733242    -101846738   42380254
                23   - 63     -26155744    -98269240   45957752
                23   - 283      2684258    -88532900   93901416
                23   - 203      6886758    -65226738   79000254
                283  - 123      -32417500    -129167907   64332907
                283  - 63     -28840002    -125590409   67910405
                283  - 23      -2684258    -93901416   88532900
                283  - 203       4202500    -92547907  100952907
                203  - 123      -36620000    -115616377   42376377
                203  - 63     -33042502    -112038879   45953875
```

```
          203  - 23       -6886758    -79000254   65226738
          203  - 283       -4202500    -100952907   92547907
                                      19:38 Wednesday, July 6, 2005   4

                    The ANOVA Procedure

          Duncan's Multiple Range Test for Extraction__ion_counts_

NOTE:  This test controls the Type I comparisonwise error rate, not the experimentwise error
                         rate.


               Alpha                        0.05
               Error Degrees of Freedom           5
               Error Mean Square          9.444E14
               Harmonic Mean of Cell Sizes 1.764706

                NOTE: Cell sizes are not equal.


      Number of Means           2           3           4           5
      Critical Range      84098089     86715580     87824543     88223977


         Means with the same letter are not significantly different.


                            Duration_
        Duncan Grouping          Mean     N    s_

               A     59267500     2    123
             A
               A     55690002     2    63
             A
               A     29534258     3    23
             A
               A     26850000     1    283
             A
               A     22647500     2    203
```

SAS R5 moist (REFER 6.2.4)

```
                                    19:38 Wednesday, July 6, 2005   1

                          The ANOVA Procedure

                         Class Level Information

                      Class        Levels    Values

                      Duration          3    23 46 109


                        Number of observations    6
                                    19:38 Wednesday, July 6, 2005   2

                          The ANOVA Procedure

    Dependent Variable: Extraction__ion_counts_   Extraction (ion counts)

                                    Sum of
      Source                  DF         Squares     Mean Square    F Value    Pr > F

      Model                    2    1.3813732E13    6.9068659E12       0.76    0.5401

      Error                    3    2.7204915E13    9.068305E12

      Corrected Total          5    4.1018647E13


           R-Square     Coeff Var      Root MSE    Extraction__ion_counts_ Mean

           0.336767      11.93765       3011363                     25225750


      Source                  DF        Anova SS     Mean Square    F Value    Pr > F

      Duration                 2    1.3813732E13    6.9068659E12       0.76    0.5401
                                    19:38 Wednesday, July 6, 2005   3

                          The ANOVA Procedure

                    t Tests (LSD) for Extraction__ion_counts_

     NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                                  rate.

                       Alpha                          0.05
                       Error Degrees of Freedom          3
                       Error Mean Square          9.068E12
                       Critical Value of t         3.18245
                       Least Significant Difference   9.58E6


                 Means with the same letter are not significantly different.


                  t Grouping          Mean      N    Duration

                           A      26527250      2    23
                           A
                           A      26052500      2    46
                           A
                           A      23097500      2    109
                                    19:38 Wednesday, July 6, 2005   4

                          The ANOVA Procedure

                  Duncan's Multiple Range Test for Extraction__ion_counts_

     NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                                  rate.

                        Alpha                     0.05
                        Error Degrees of Freedom     3
                        Error Mean Square     9.068E12
```

```
        Number of Means          2          3
        Critical Range      9583515     9615390

  Means with the same letter are not significantly different.

Duncan Grouping         Mean     N   Duration

          A      26527250      2    23
        A
          A      26052500      2    46
        A
          A      23097500      2    109
```

SAS R5-Dry (REFER 6.2.5)

```
                                 19:38 Wednesday, July 6, 2005   1

                         The ANOVA Procedure

                        Class Level Information

                  Class          Levels    Values

                  Duration_s_         4    10 30 90 270


                       Number of observations    4
                                 19:38 Wednesday, July 6, 2005   2

                         The ANOVA Procedure

Dependent Variable: Extraction__ion_counts_   Extraction (ion counts)

                               Sum of
Source                DF      Squares    Mean Square    F Value    Pr > F

Model                  3     6.075E15       2.025E15          .         .

Error                  0            0              .

Corrected Total        3     6.075E15


      R-Square     Coeff Var     Root MSE    Extraction__ion_counts_ Mean

      1.000000             .            .                      1137500000


Source                DF      Anova SS    Mean Square    F Value    Pr > F

Duration_s_            3     6.075E15       2.025E15          .         .
                                 19:38 Wednesday, July 6, 2005   3

                         The ANOVA Procedure

               Level of          ---Extraction__ion_counts_---
               Duration_s_    N          Mean          Std Dev

               10             1    1190000000                .
               30             1    1140000000                .
               90             1    1080000000                .
               270            1    1140000000                .
                                 19:38 Wednesday, July 6, 2005   4

                         The ANOVA Procedure

               Level of          ---Extraction__ion_counts_---
               Duration_s_    N          Mean          Std Dev

               10             1    1190000000                .
               30             1    1140000000                .
               90             1    1080000000                .
               270            1    1140000000                .
```

SAS analysis of dry Pepper triplicates (REFER 6.3)

```
                                   16:50 Wednesday, July 6, 2005   1

                         The ANOVA Procedure

                        Class Level Information

      Class        Levels    Values

      Field           10    81.1 81.83 81.84 83.19 84.56 85.32 86.83 87.41 92.14 93.46


                       Number of observations    32
                                   16:50 Wednesday, July 6, 2005   2

                         The ANOVA Procedure

   Dependent Variable: Extraction   Extraction

                               Sum of
      Source                DF         Squares     Mean Square    F Value    Pr > F

      Model                  9    4.5185102E17    5.0205669E16       2.05    0.0815

      Error                 22    5.3808704E17    2.4458502E16

      Corrected Total           31    9.8993806E17


             R-Square     Coeff Var      Root MSE    Extraction Mean

             0.456444      12.79978     156392141         1221834381


      Source                DF       Anova SS     Mean Square    F Value    Pr > F

      Field                  9    4.5185102E17    5.0205669E16       2.05    0.0815
                                   16:50 Wednesday, July 6, 2005   3

                         The ANOVA Procedure

                  Duncan's Multiple Range Test for Extraction

    NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                           rate.


                    Alpha                         0.05
                    Error Degrees of Freedom          22
                    Error Mean Square           2.446E16
                    Harmonic Mean of Cell Sizes 3.157895

                      NOTE: Cell sizes are not equal.


    Number of Means           2           3           4           5           6
    Critical Range      258115290     271024953     279276194     285075565     289381483

        Number of Means           7           8           9          10
        Critical Range      292690337     295297673     297385699     299077366


            Means with the same letter are not significantly different.


               Duncan Grouping         Mean      N    Field

                     A          1419856078      4    83.19
                   A
                 B   A          1286963808      3    81.84
               B   A
                 B   A          1285458544      3    92.14
               B   A
                 B   A          1284409327      3    87.41
               B   A
                 B   A   C      1267935507      3    86.83
               B   A   C
                 B   A   C      1218930386      3    93.46
```

```
           B   A   C
            B   A   C    1205692573     3    84.56
           B   A   C
            B   A   C    1153723086     3    81.83
           B       C
            B       C   1086445179     4    85.32
                   C
                    C   988051826     3    81.1
                                    16:50 Wednesday, July 6, 2005   4

                    The ANOVA Procedure

                  t Tests (LSD) for Extraction

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                          rate.


              Alpha                     0.05
              Error Degrees of Freedom        22
              Error Mean Square        2.446E16
              Critical Value of t       2.07387


       Comparisons significant at the 0.05 level are indicated by ***.


                  Difference
     Field          Between
   Comparison          Means    95% Confidence Limits

   83.19 - 81.84     132892270   -114824549  380609088
   83.19 - 92.14     134397533   -113319285  382114352
   83.19 - 87.41     135446751   -112270068  383163569
   83.19 - 86.83     151920570    -95796248  399637389
   83.19 - 93.46     200925692    -46791127  448642510
   83.19 - 84.56     214163505    -33553314  461880323
   83.19 - 81.83     266132992     18416173  513849810  ***
   83.19 - 85.32     333410899    104069689  562752108  ***
   83.19 - 81.1     431804252    184087433  679521070  ***
   81.84 - 83.19    -132892270   -380609088  114824549
   81.84 - 92.14       1505263   -263315155  266325681
   81.84 - 87.41       2554481   -262265937  267374899
   81.84 - 86.83      19028300   -245792118  283848718
   81.84 - 93.46      68033422   -196786996  332853840
   81.84 - 84.56      81271235   -183549183  346091653
   81.84 - 81.83     133240722   -131579696  398061140
   81.84 - 85.32     200518629    -47198189  448235447
   81.84 - 81.1     298911982     34091564  563732400  ***
   92.14 - 83.19    -134397533   -382114352  113319285
   92.14 - 81.84      -1505263   -266325681  263315155
   92.14 - 87.41       1049217   -263771201  265869635
   92.14 - 86.83      17523037   -247297381  282343455
   92.14 - 93.46      66528158   -198292260  331348576
   92.14 - 84.56      79765972   -185054446  344586390
   92.14 - 81.83     131735458   -133084960  396555876
   92.14 - 85.32     199013366    -48703453  446730184
   92.14 - 81.1     297406718     32586300  562227136  ***
   87.41 - 83.19    -135446751   -383163569  112270068
   87.41 - 81.84      -2554481   -267374899  262265937
   87.41 - 92.14      -1049217   -265869635  263771201
   87.41 - 86.83      16473820   -248346598  281294238
   87.41 - 93.46      65478941   -199341477  330299359
   87.41 - 84.56      78716754   -186103664  343537172
   87.41 - 81.83     130686241   -134134177  395506659
   87.41 - 85.32     197964148    -49752670  445680967
   87.41 - 81.1     296357501     31537083  561177919  ***
                                    16:50 Wednesday, July 6, 2005   5

                    The ANOVA Procedure

                  t Tests (LSD) for Extraction

       Comparisons significant at the 0.05 level are indicated by ***.


                  Difference
```

```
  Field        Between
 Comparison        Means   95% Confidence Limits

 86.83 - 83.19  -151920570   -399637389  95796248
 86.83 - 81.84   -19028300  -283848718 245792118
 86.83 - 92.14   -17523037  -282343455 247297381
 86.83 - 87.41   -16473820  -281294238 248346598
 86.83 - 93.46    49005121  -215815297 313825539
 86.83 - 84.56    62242935  -202577483 327063353
 86.83 - 81.83   114212421   -150607997 379032839
 86.83 - 85.32   181490329    -66226490 429207147
 86.83 - 81.1   279883681     15063263 544704099  ***
 93.46 - 83.19  -200925692   -448642510  46791127
 93.46 - 81.84   -68033422  -332853840 196786996
 93.46 - 92.14   -66528158  -331348576 198292260
 93.46 - 87.41   -65478941  -330299359 199341477
 93.46 - 86.83   -49005121  -313825539 215815297
 93.46 - 84.56    13237813  -251582605 278058231
 93.46 - 81.83    65207300  -199613118 330027718
 93.46 - 85.32   132485207   -115231611 380202026
 93.46 - 81.1   230878560    -33941858 495698978
 84.56 - 83.19  -214163505   -461880323  33553314
 84.56 - 81.84   -81271235  -346091653 183549183
 84.56 - 92.14   -79765972  -344586390 185054446
 84.56 - 87.41   -78716754  -343537172 186103664
 84.56 - 86.83   -62242935  -327063353 202577483
 84.56 - 93.46   -13237813  -278058231 251582605
 84.56 - 81.83    51969487  -212850931 316789905
 84.56 - 85.32   119247394   -128469424 366964212
 84.56 - 81.1   217640747    -47179671 482461165
 81.83 - 83.19  -266132992   -513849810 -18416173  ***
 81.83 - 81.84  -133240722   -398061140 131579696
 81.83 - 92.14  -131735458   -396555876 133084960
 81.83 - 87.41  -130686241   -395506659 134134177
 81.83 - 86.83  -114212421   -379032839 150607997
 81.83 - 93.46   -65207300  -330027718 199613118
 81.83 - 84.56   -51969487  -316789905 212850931
 81.83 - 85.32    67277907  -180438911 314994726
 81.83 - 81.1   165671260    -99149158 430491678
 85.32 - 83.19  -333410899   -562752108 -104069689  ***
 85.32 - 81.84  -200518629   -448235447  47198189
 85.32 - 92.14  -199013366   -446730184  48703453
 85.32 - 87.41  -197964148   -445680967  49752670
 85.32 - 86.83  -181490329   -429207147  66226490
 85.32 - 93.46  -132485207   -380202026 115231611
 85.32 - 84.56  -119247394   -366964212 128469424
 85.32 - 81.83   -67277907  -314994726 180438911
 85.32 - 81.1    98393353   -149323466 346110171
 81.1  - 83.19  -431804252   -679521070 -184087433  ***
                                16:50 Wednesday, July 6, 2005   6

              The ANOVA Procedure

           t Tests (LSD) for Extraction

Comparisons significant at the 0.05 level are indicated by ***.


          Difference
  Field        Between
 Comparison        Means   95% Confidence Limits

 81.1  - 81.84  -298911982   -563732400 -34091564  ***
 81.1  - 92.14  -297406718   -562227136 -32586300  ***
 81.1  - 87.41  -296357501   -561177919 -31537083  ***
 81.1  - 86.83  -279883681   -544704099 -15063263  ***
 81.1  - 93.46  -230878560   -495698978  33941858
 81.1  - 84.56  -217640747   -482461165  47179671
 81.1  - 81.83  -165671260   -430491678  99149158
 81.1  - 85.32   -98393353  -346110171 149323466
```

SAS Coriander (REFER 6.4)

```
                                   19:38 Wednesday, July 6, 2005   1

                         The ANOVA Procedure

                        Class Level Information

               Class         Levels    Values

               Field__V_m_        5    81.1 84.56 86.83 87.41 92.14


                        Number of observations    15
                                   19:38 Wednesday, July 6, 2005   2

                         The ANOVA Procedure

        Dependent Variable: Cumulative   Cumulative

                                      Sum of
          Source              DF         Squares     Mean Square    F Value    Pr > F

          Model                4    6.4949176E13    1.6237294E13       3.44    0.0513

          Error               10    4.7155983E13    4.7155983E12

          Corrected Total     14    1.1210516E14


               R-Square     Coeff Var      Root MSE    Cumulative Mean

               0.579359      8.623233       2171543           25182467


          Source              DF        Anova SS     Mean Square    F Value    Pr > F

          Field__V_m_          4    6.4949176E13    1.6237294E13       3.44    0.0513
                                   19:38 Wednesday, July 6, 2005   3

                         The ANOVA Procedure

                        t Tests (LSD) for Cumulative

 NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                                  rate.


                      Alpha                             0.05
                      Error Degrees of Freedom            10
                      Error Mean Square             4.716E12
                      Critical Value of t            2.22814
                      Least Significant Difference    3.95E6


                 Means with the same letter are not significantly different.


                                        Field__
                 t Grouping      Mean    N   V_m_

                          A    26892333    3   81.1
                          A
                          A    26657333    3   84.56
                          A
                          A    25915667    3   92.14
                          A
                          A    25265333    3   86.83

                          B    21181667    3   87.41
                                   19:38 Wednesday, July 6, 2005   4

                         The ANOVA Procedure

                    Duncan's Multiple Range Test for Cumulative

 NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                                  rate.
```

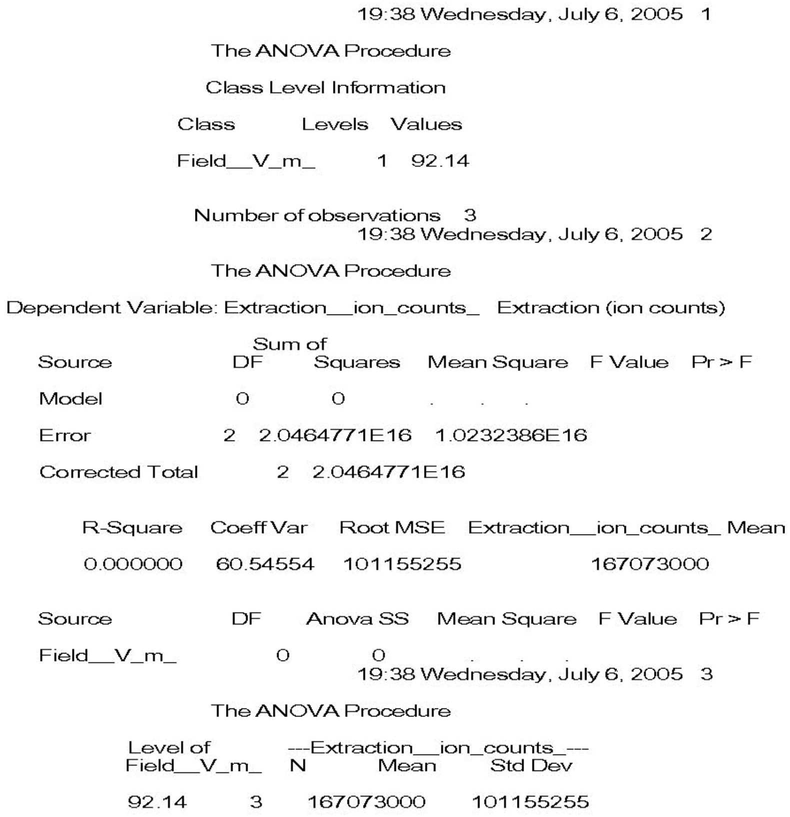
```
          Alpha                    0.05
          Error Degrees of Freedom       10
          Error Mean Square        4.716E12

Number of Means           2          3          4          5
Critical Range      3950607     4128359     4232984     4299912

   Means with the same letter are not significantly different.

                               Field__
 Duncan Grouping                Mean     N   V_m_

          A      26892333      3   81.1
        A
          A      26657333      3   84.56
        A
          A      25915667      3   92.14
        A
          A      25265333      3   86.83

          B      21181667      3   87.41
```

SAS Low moisture triplicates (REFER 6.6.1)

```
                                 19:38 Wednesday, July 6, 2005   1

                         The ANOVA Procedure

                        Class Level Information

              Class          Levels    Values

              Field_V_m_          5    58.64 81.84 84.56 85.32 92.14


                        Number of observations    14
                                 19:38 Wednesday, July 6, 2005   2

                         The ANOVA Procedure

 Dependent Variable: Extraction   Extraction

                                   Sum of
   Source                    DF        Squares     Mean Square    F Value    Pr > F

   Model                      4    1.6608101E15    4.1520253E14      1.22    0.3675

   Error                      9    3.0625814E15    3.4028683E14

   Corrected Total           13    4.7233915E15


              R-Square     Coeff Var      Root MSE    Extraction Mean

              0.351614      78.52307      18446865           23492286


   Source                    DF       Anova SS     Mean Square    F Value    Pr > F

   Field_V_m_                 4   1.6608101E15    4.1520253E14      1.22    0.3675
                                 19:38 Wednesday, July 6, 2005   3

                         The ANOVA Procedure

                 Duncan's Multiple Range Test for Extraction

 NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
       rate.


                   Alpha                           0.05
                   Error Degrees of Freedom           9
                   Error Mean Square           3.403E14
                   Harmonic Mean of Cell Sizes 1.764706

                    NOTE: Cell sizes are not equal.


   Number of Means          2           3           4           5
   Critical Range    44423090    46368252    47487869    48185425


         Means with the same letter are not significantly different.


                                     Field_
      Duncan Grouping          Mean      N    V_m_

                    A      36061000      6    58.64
                    A
                    A      14724000      1    92.14
                    A
                    A      14306000      3    81.84
                    A
                    A      14012000      3    84.56
                    A
                    A      12848000      1    85.32
                                 19:38 Wednesday, July 6, 2005   4

                         The ANOVA Procedure
```

```
                    t Tests (LSD) for Extraction

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error
                           rate.


                   Alpha                    0.05
                   Error Degrees of Freedom       9
                   Error Mean Square       3.403E14
                   Critical Value of t      2.26216


            Comparisons significant at the 0.05 level are indicated by ***.


                  Difference
   Field_V_m_        Between
   Comparison         Means   95% Confidence Limits

   58.64 - 92.14    21337000    -23736236  66410236
   58.64 - 81.84    21755000     -7752359  51262359
   58.64 - 84.56    22049000     -7458359  51556359
   58.64 - 85.32    23213000    -21860236  68286236
   92.14 - 58.64   -21337000    -66410236  23736236
   92.14 - 81.84      418000   -47767316  48603316
   92.14 - 84.56      712000   -47473316  48897316
   92.14 - 85.32     1876000    -57138718  60890718
   81.84 - 58.64   -21755000    -51262359   7752359
   81.84 - 92.14     -418000   -48603316  47767316
   81.84 - 84.56      294000   -33778164  34366164
   81.84 - 85.32     1458000    -46727316  49643316
   84.56 - 58.64   -22049000    -51556359   7458359
   84.56 - 92.14     -712000   -48897316  47473316
   84.56 - 81.84     -294000   -34366164  33778164
   84.56 - 85.32     1164000    -47021316  49349316
   85.32 - 58.64   -23213000    -68286236  21860236
   85.32 - 92.14    -1876000    -60890718  57138718
   85.32 - 81.84    -1458000    -49643316  46727316
   85.32 - 84.56    -1164000    -49349316  47021316
```

SAS High moisture (REFER 6.6.2)



```
                                       19:38 Wednesday, July 6, 2005   1

                          The ANOVA Procedure

                         Class Level Information

                      Class          Levels    Values

                      Field__V_m_         1    92.14

                        Number of observations    3
                                       19:38 Wednesday, July 6, 2005   2

                          The ANOVA Procedure

  Dependent Variable: Extraction__ion_counts_   Extraction (ion counts)

                                 Sum of
     Source                DF        Squares     Mean Square    F Value    Pr > F

     Model                  0              0           .          .          .

     Error                  2    2.0464771E16    1.0232386E16

     Corrected Total        2    2.0464771E16

         R-Square     Coeff Var      Root MSE    Extraction__ion_counts_ Mean

         0.000000      60.54554     101155255                       167073000

     Source                DF       Anova SS     Mean Square    F Value    Pr > F

     Field__V_m_            0              0           .          .          .
                                       19:38 Wednesday, July 6, 2005   3

                          The ANOVA Procedure

                 Level of           ---Extraction__ion_counts_---
                 Field__V_m_    N           Mean          Std Dev

                 92.14          3      167073000        101155255
```