Microwave Assisted Extraction of Non-Volatiles from Ginger using Ionic Liquids

K. K. Saranya, K. Ramalakshmi, L. Jagan Mohan Rao

Abstract: Conditions were optimised for the extraction of non-volatiles from ginger using ionic liquids employing microwave energy. Maximum extraction could be achieved with the ionic liquid namely [BMIM]Cl (1-methyl -3-imidazolium chloride) at the concentration of 0.3 M with material to solvent ratio at 1:6 employing the microwave power of 600W, time (5 min.) and temperature (70°C). Microwave assisted extraction using ionic liquid was compared with that of traditional methods with respect to yield and total polyphenolic content (TPP) as well as radical scavenging activity (RSA). Increase in gingerol content (30%) was observed in microwave assisted ionic liquid based extraction than in the conventional extraction. Yield of extract increased by 3.5 fold with optimized condition than the traditional method. Optimized method for the extraction of ginger gives 38.25% increase in the RSA value and 71.93% increase in TPP content compared to the traditional method. The gingerol content released into extract increased by 4.5 folds under optimized condition than the traditional method. It is concluded that microwave assisted ionic liquid extraction can be adopted for the extraction of non-volatiles from ginger and can be extended to the industrial scale also.

Keywords: Microwave, Ionic liquid, Ginger, Non volatiles

I. INTRODUCTION

Ginger (*Zingiber officinale* Rosc) is a spice belongs to the family *Zingiberaceae* and the rhizomes are used in the culinary preparations. India is one of the largest producers of fresh as well as dry ginger. Other major producers of ginger in the world are West Indies, Brazil, China, Japan and Indonesia. In India, the major ginger producing states are Kerala, Orissa, Andhra Pradesh, Himachal Pradesh, Meghalaya and West Bengal.

A. Health benefits of ginger

Fresh and dry ginger has been used in Indian traditional medicine for relief from the diseases such as arthritis, rheumatism, sprains, muscular aches and pains, congestion, coughs, sinusitis, sore throats, diarrhoea, indigestion, loss of appetite, fever, flu. Gingerols, Recent report reveals that extracts with ethyl acetate of ginger produced a significant reduction in elevated lipid levels and body weight. The activity appears to be dependent on the concentration of [6]-gingerol present in the extracts [3,4]. The essential oils of ginger showed good anticancer and anti-inflammatory properties[5,6,7,8] which is mainly due to the compounds such as Zingiberene, β-sesquiphellandrene and ar-curcumene.

B. Chemistry of ginger components

It is well known that the physico-chemical composition varies based on the source location and environment of the rhizomes. The odour of ginger depends mainly on volatile oil and its yield varies from 1 to 3%. More than 50 components of the oil are characterized which includes monoterpenoids [β-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol] and sesquiterpenoids [α-zingiberene (30-70%), β-sesquiphellandrene (15-20%), β-bisabolene (10-15%), (E-E)-α-farnesene, ar-curcumene, zingiberol]. Dehydration of ginger converts some intense oil components into less odour-defining compounds [9].

Due to the gingerols, pungency of fresh ginger is a homologous series of phenols. Even though other gingerols with various chain lengths are present, [6]-gingerol is the popular and frequently used. The pungency of dry ginger is shogaols (i.e., [6]-shogaol), that are dehydrated methods of gingerols. In thermal processing, Shogaols are made from the corresponding gingerols [10]. Jolad *et al* (2004) found 63 compounds [11] from organically-grown fresh ginger which includes gingerols, shogaols, paradols, dihydroparadols and acetyl derivatives of gingerols (Figure 1.) and reported that thermal degradation from gingerols to ginger, shogaols, and associated compounds. Jolad et *al*. (2005) [12, 13] reported [6]-, [8]-, [10]- and [12]-ginger diones along with 12 known compounds.

C. Microwave assisted extraction of ginger

Canada's Federal Department of the Environment developed and patented Microwave-Assisted Process (MAP^{TM})[14,15,16]. It is commonly utilized to extract soluble materials from different matrices and commonly used an organic solvents [17,18,19,20].

In liquid-phase microwave- assisted extraction, the matrix containing the target components are immersed in a solvent is apparent or partly apparent to microwaves and the matrix is selectively heated. It is used in industries and laboratories.

They are tremendously speedy and signified through extremely sensitivity, and enhanced yields. Solvent consumption minimized energy level, and provides better selectivity.

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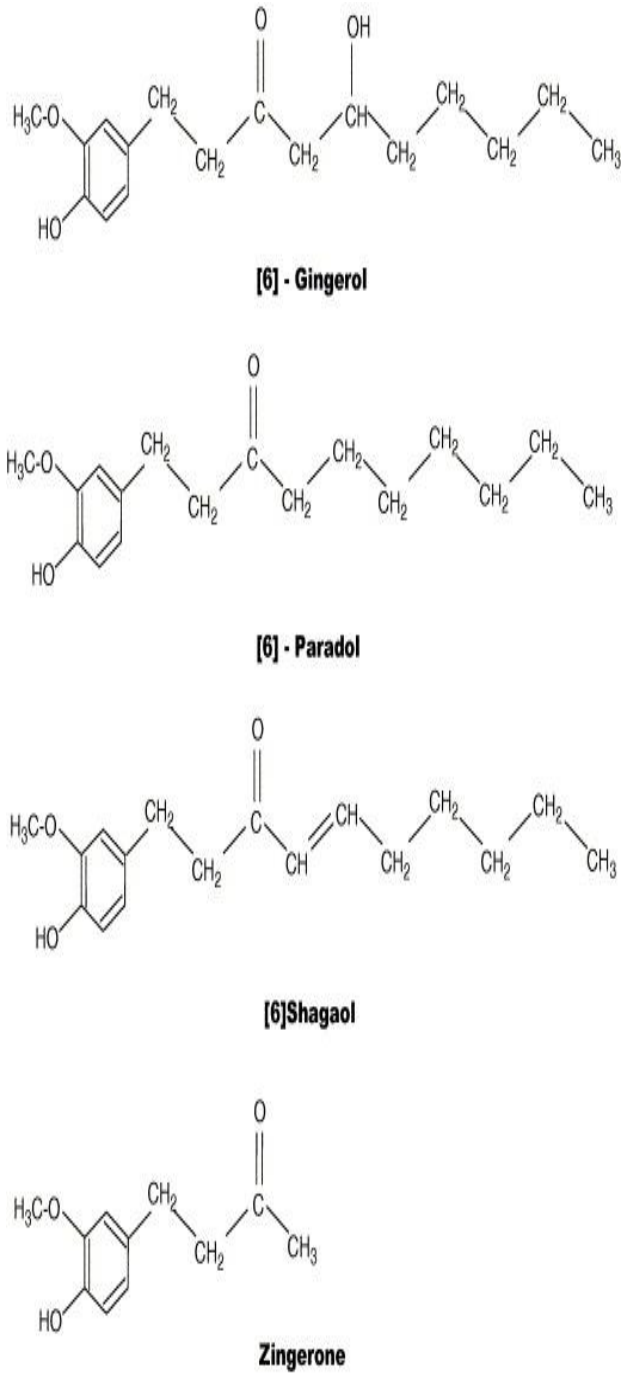

Figure 1. Major components of ginger.

D. Ionic liquid (IL) based microwave assisted extraction of ginger

Microwave-assisted extraction (MAE) has already been widely applied to the extraction of active constituents in plants. The polarity of the solvent is very important in microwave extraction (ME). Polar solvents such as water, methanol and acetone are generally used since these can absorb more microwave energy [21]. Non-polar solvent was also used in microwave-assisted extraction. The properties of IL such as non-flammability, low vapour pressure, high specific solvent abilities, chemical and thermal stabilities and ease of recycling and manipulation provided lot many applications in various fields such as food application,

heavy metal removal, solar thermal energy etc. ILs for the extraction of spices could overcome most of the above difficulties because it doesn't need high temperature and it can simplify the whole system. The extraction can be done without the pre-treatment of the sample if ionic liquid is used as extraction medium. The usage of IL (as absorption medium) in conjunction with MW method has shown effective extraction of spices. The extraction efficiency of spices using IL depends mainly on the concentration of IL, irradiation power, solid-liquid ratio, extraction time, alkyl substituent present, properties of IL (viscosity, miscibility etc) and nature of cation or anion of IL etc.

II. MATERIALS AND METHODS

A. Reagents and chemicals

The solvents used for the extraction include 1-butyl-3-methylimidazolium chloride (BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl),1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF_4) and water. The other reagents used are methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent (FC reagent), sodium carbonate.

B. Raw materials

Fresh rhizomes (10 kg) of *Zingiber officinale* Rosc is procured from the supplier in Mysore, India. The rhizomes are pre-treated, cleaned with water and carved. Exhausting a Tray dryer at 50°C for six hours and crushed to powder, the slices are exposed to dehydration.

C. Microwave extraction

Dried ginger powder (1g) was extracted in a closed microwave system (Model: STARTS configuration with Milestone, Italy control terminal 260; Built in focused magnetic stirrer and IR sensor; Frequency- 50 Hz; Magnetron: SN: 133613;) using water with various ionic liquids such as [BMIM]Cl, [EMIM]Cl, [BMIM]BF_4 under different set of conditions with respect to time (2, 5 and 7 min), wattage (200, 400, 600,800 W) and temperatures (50, 70, 90 ºC). After extraction, the sample dispersion was filtered and the filtrate is used for the estimation of yield, RSA, TPP and [6]-gingerol content. The experimental details are furnished in Table 1.

Ginger powder (1g) was also extracted using water at optimized conditions using microwave energy. Ginger powder (1g) was also extracted using ionic liquid [BMIM]Cl at optimized conditions. It was kept for magnetic stirring at optimum temperature and time in a magnetic stirring machine. These are subjected to filtration and the filtrate is used for the estimation of yield, RSA, TPP and [6]-gingerol content. The results were compared with the ionic liquid based microwave assisted extraction of ginger.

Table 1: Microwaves assisted extraction of ginger using ionic liquids

Exp set	Variable parameter	Fixed parameter	Variables
1	Solvent	Temperature (90ºC) Wattage (800W) Time (5 min) Material to solvent (1:8) Molarity (0.2M)	[BMIM] Cl [EMIM] Cl, [BMIM] BF4 Water
II	Material to Solvent	[BMIM] Cl Temperature (90ºC) Wattage (800W) Time (5 min) Molarity (0.2M)	1:2 1:6 1:8
III	Concentration (M)	Material to solvent (1:6) [BMIM] Cl Temerature (90ºC) Wattage (800W) Time (5 min)	0.1 0.2 0.3
IV	Time (min)	Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Temperature (90ºC) Wattage (800W)	2 5 7
V	Temperature (90ºC)	Time (7 min) Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Wattage (800W)	50 70 90
	Wattage (W)	Temperature (70 ºC) Time (7 min) Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Wattage (800W	200 400 600 800

D. Total polyphenols estimation

Total polyphenol (TPP) content of extracts was determined using FC reagent by Swain & Hillis, (1959) [22]. The extract (1 mL) was diluted with water (100 mL). 0.5 mL of FC reagent and 1.5 ml of saturated sodium carbonate solution are mixed to the 0.5ml of sample solution and then prepared up to 10 mL with water. In room temperature for sixty minutes, the solution was incubated and absorbance is measured at 765 nm. Gallic acid stock solution (0.01%) and working solutions (20-200 µg/10 mL) were prepared. These solutions were treated as above and the absorbance was measured. The standard curve was drawn using gallic acid concentration versus absorbance. The over-all polyphenol quantity of extract is represented as Gallic acid equivalents.

E. Radical scavenging activity

As per the process of Blois and Jayaprakasha & Jagan Mohan Rao, radical scavenging activity (RSA) of various extracts is estimated [23, 24]. 1 ml of Sample solution for various extracts at 10, 20 and 30 ppm of concentrations level are mixed with 0.1 mM and 4 mL of methanolic solution of DPPH and permitted for twenty minutes in 27 ºC. Methanol is applied for standard correction and optical density (OD) of the samples estimated at 517 nm. Radical scavenging activity is represented as the inhibition (%) of free radical and it is computed as:

$$\% \text{ inhibition} = \frac{100 \times (\text{control OD} - \text{sample OD})}{\text{control OD}}$$

F. Estimation of [6]-gingerol using chromatography

The ginger extracts were analyzed on a High Performance Liquid Chromatograph system (Make: Shimadzu : LC-10AT) is armed with a Diode Array Detector (SPD-M 10A VP)and controller (SCL-10A VP). Chromatographic analysis was carried out with the mobile phase of acetonitrile-water (55:45, v/v) using a Waters Spherisorb C-18 (ODS 2, 4.6 mm×250 mm, 5 µm). HPLC operating factors such as flow rate-1.0 mL/min and injection volume-10 µL. At the wavelength of 280 nm, eluting compounds found [25]. Extracts are softened in methanol and inoculated. Replica injections are passed for ensuring the reproducibility and exactness. At several concentrations retaining the identical condition, Calibration curve of gingerol is built. Using the calibration curve, [6]-gingerol content of the samples is detected.

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III. RESULTS AND DISCUSSION

A. Selection of ionic liquid

Microwave extraction of the ginger powder using water and various ionic liquids such as [BMIM]Cl, [EMIM]Cl, [BMIM]BF_4 is carried out and the results of total polyphenol and radical scavenging activity of the extracts along with yield are given in Table 2. From the result, it is clear that the ionic liquid [BMIM]Cl (1-methyl -3-imidazolium chloride) gives better yield of extraction than any other solvents selected including water.

Hence, it is selected as the solvent for further studies. Lei Yang *et al (* 2012) [26] extracted proanthocyanidins from Larixgmelini Bark using 1-butyl-3-methylimidazolium bromide and it was found to be the main contributor to proanthocyanidins extraction than water in the ionic liquid–water system. The variation in yield of extract was observed with different ionic liquids. [BMIM]Cl provided better extraction than with water which indicates that the microwave absorption performance of IL is better than water. The extract obtained using [BMIM]Cl showed higher TPP and RSA.

B. Influence of solvent ratio and the concentration on extraction

The material to solvent ratio is an important factor and was studied to optimize extraction yield. This ratio has a major influence on the extraction efficiency [26]. By keeping the molarity (0.2M), Time (5 min) and temperature (880W) constant, different solvent to material ratio was taken and the extraction was carried out. Similarly, the concentration of the ionic liquid was also optimised.

A series of experiments were done with various materials to solvent ratios (1:4, 1:6, 1:8) to optimise the same. If material is kept as a solvent ratio at 1:6 and [BMIM]Cl as a extracting solvent, maximum extraction is achieved. However, when the solvent ratio changed to 1:6 from 1:4 there is a tremendous increase in the RSA and TPP values, but if transformed from 1:6 to 1:8, higher solvent volumes didn't pointedly transformed the RSA values whereas the TPP content decreased marginally. Therefore, a material to solvent ratio of 1:6 was optimised and used for further experiments.

The extraction procedures were performed using [BMIM]Cl solution of different concentrations (0.1 to 0.3 M) to determine the optimum concentration for extraction employing microwave energy and results are presented (Table 2). It is clear that the extraction yield increased when the [BMIM] Cl concentration increased from 0.1to 0.3 M. With the addition of [BMIM] Cl, both extraction capacity of the solvent and solubility are enhanced. When concentration changes from 0.1M to 0.2M, increase in RSA value is observed and it further increases with rise in concentration. The increase in the TPP values are gradual according to the increase in the concentration of the ionic liquid. Finally, 0.3M [BMIM]Cl solution is selected as an optimal ionic liquid concentration.

Table 2. Quality parameters of ginger extracts using ionic liquids

Variable parameter	Fixed parameter	Variables	% yield	RSA	TPP as gallic acid equivalents (mg/g)
Solvent	Temperature (90ºC) Wattage (800W) Time (5 min) Material to solvent (1:8) Molarity (0.2M)	[BMIM] Cl	4.49	13.28	2.65
		[EMIM] Cl,	4.15	11.97	2.58
		[BMIM] BF4	4.10	12.22	2.55
		Water	4.20	10.83	2.44
Material to Solvent	[BMIM] Cl Temperature (90ºC) Wattage (800W) Time (5 min) Molarity (0.2M)	1:4	4.10	7.86	3.53
		1:6	4.53	19.04	3.55
		1:8	4.50	18.45	3.53
Concentration (M)	Material to solvent (1:6) [BMIM] Cl Temerature (90ºC) Wattage (800W) Time (5 min)	0.1	4.31	19.74	1.79
		0.2	4.52	20.45	2.16
		0.3	5.49	20.75	2.59
Time (min)	Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Temperature (90ºC) Wattage (800W)	2	2.88	21.80	2.25
		5	4.85	23.46	2.70
		7	2.76	22.91	2.64
Temperature (90ºC)	Time (5 min) Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Wattage (800W)	50	3.70	22.06	3.96
		70	3.53	23.86	4.50
		90	2.66	23.39	3.72

Wattage (W)	Temperature (70 ºC) Time (5 min) Concentration (0.3M) Material to solvent (1:6) [BMIM] Cl Wattage (800W)	200	4.11	23.78	3.24
		400	4.157	24.23	2.91
		600	4.370	24.74	4.91
		800	4.412	23.80	4.45

C. Influence of time on extraction

To optimize exposure time, extractions were carried out at 800 W microwave power with various exposure times starting from 2 to 7min and the results are shown in Table 2. It is noticed that extraction yield is improved if microwave exposure time is stretched from two to five minutes.

Table 3: Comparison of ginger extraction of non-volatiles under optimized condition with conventional extraction

Method of extraction	**Yield (%)**	**RSA (%)**	**TPP (µg GAE /g of sample)**	**[6]-gingerol content (mg%)**
Ionic liquid based microwave extraction with optimized condition	5.47	24.719	4.9	3.64x10-5
Extraction without microwave power, but IL as solvent	3.13	18.29	3.01	1.20x10-5
Extraction without ionic liquid and microwave power	1.565	17.88	2.85	0.813x10-5

Extended application of microwaves (more than 5 min) resulted in decrease of extraction yield. There is a change RSA values according to the exposure time. It is clear that RSA values showed increase when exposure time increased from 2 to 5 min and decrease was observed when time changed to 7 min. The TPP values increased with increase in the exposure time up to 5 min and decreased from 5 to 7 min. Therefore, 5 min was set for all subsequent experiments. Considering the improvement of extraction efficiency and the relatively shorter extraction time, 5 min were selected as the appropriate extraction time.

D. Influence of temperature on extraction

The extraction temperature generally has an influence on the recovery of bioactive ingredients [27]. Greater the temperature of extraction medium, higher the diffusivity of the solvent into cells and desorption and solubility of compounds from the cells, which results in more yield of components [28, 29], although some bioactive compounds from plants could be decomposed at an elevated temperature [30].

The extraction efficiency shows a slight decrease with the temperature elevated from 50^{o}C to 70^{o}C and it decreases rapidly when it reaches 70^{o}C (Table 2). The RSA values shows rise according to increase in the temperature from 50^{o}C to 70^{o}C and later it showed decrease in the value. In the case of TPP content, values shows gradual increase(50 - 70^{o}C) and decrease (70 - 90^{o}C). Thus, 70 °C was chosen as the optimal extraction temperature.

E. Influence of microwave power on extraction

Microwave power is a significant factor in microwave assisted extraction. Experiments are tested at 200,400, 600 and 800 to examine the microwave power's effect on the extraction yield, by preserving MAE time constant as five minutes.

When the irradiation power was increased from 200 to 800 W, the extraction yield is enhanced. If irradiation power is maximized from 200W to 600 W, rapid increase in the TPP values is observed where as RSA values increases gradually. According to increase in the microwave power above 600 W, the TPP and RSA values decreased instead. Though ionic liquid has absorbing power for microwave energy, better power can causes the plant to be burned by the ruin the vital components, while lesser power might lead a longer time of extraction of the target compounds [26] (Lei Yang et.al, 2012). Therefore, irradiation power of MAE is kept at 600 W in the consequent trials.

F. Comparative analysis of MAE and conventional extracts

Microwave assisted extracts using ionic liquid was compared with the extracts obtained using traditional methods with respect to the values obtained for radical scavenging activity (RSA), polyphenol content and total yield of the extract. These are indispensable in order to analyze the efficiency of the suggested method. The comparison of the three methods is given below in the table 3. Yield of extract incresed by 3.5 fold with optimized condition (Table 3) than the traditional method.The extract prepared with the use of ionic liquid (without microwave power) shows 100% increse in yield than traditional method. Optimized method for the extraction of ginger gives 38.25% increase in the RSA value and 71.93% increase in TPP content compared to the traditional method. Extracts with ionic liquid without microwave radiation also caused increase in the RSA and TPP values. The gingerol content obtained from the extracts of various conditions such as the extract where only water is used as the solvent, extract with ionic liquid as the solvent prepared without the application of microwave and extract using microwave energy and ionic liquid as solvent (optimized condition) were also presented in Table 3.

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The gingerol content released into extract increased by 4.5 folds under optimized condition (Table 3) than the traditional method. The extract prepared with ionic liquid as solvent (without microwave power) showed a slight improvement (1.5 fold) in the gingerol content than traditional method.

IV. SUMMARY AND CONCLUSION

MAE is used to elimination of dynamic portions in ginger using ethanol, methanol and so on as solvents. The optimized parameters for the extraction are set on the basis of yield of the extract, TPP as well as radical scavenging activity. The optimized condition for the effective extraction of ginger are: material to solvent ratio - 1:6, the microwave power - 600W, time - 5min, temperature - 70oC and the concentration of the ionic liquid solution - 0.3M. The outcome specifies microwave absorption performance of IL is improved than water and if IL is replaced for water to absorb microwave energy, extraction time will be minimized considerably, since IL takes great capacity for absorbing microwave. On comparison with the suggested method with the conventional method it is clear that the extract prepared by optimized method provides good improvement in all the parameters in particular gingerol content. This is mainly due to the application of ionic liquid and microwave. Ionic liquid can absorb microwaves than any other solvents including water, ethanol etc. and microwave can penetrate the cells which will result in effective extraction by reducing the time of extraction, amount of solvent to be used and temperature

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