Inactivation of Contaminated Fungi in Rice Grains by Dielectric Heating

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Abstract: The quality of rice is decreased when contaminated with fungi. Aspergillus species are the most frequently found in rice. This research proposes using a dielectric heating method for fungal inactivation in rice grains by radio frequency (RF) energy. In order to understand the interaction between the fungi contaminating rice and electrical energy, dielectric properties comparison between Aspergillus sp. BP17 and rice powder were measured using an open-ended coaxial probe with a vector network analyzer (VNA) to develop dielectric heating equipment. The effect of RF energy on the dielectric heating system (9 kW, 40.68 MHz) is investigated based on different electric field intensities (150, 190, 225, 300, and 450 kV/m) with different temperatures (70, 80, 90, 100, and 110 °C). The growth of fungi contaminating rice was determined using a direct count method and reported as a percentage of inactivation. The result showed that the fungal inactivation of 100% was obtained at the electric field intensity value ≥ 225 kV/m at the lowest temperature of 90 °C. The combination of temperature and electric field intensity significantly (p ≤ 0.05) increased the percentage of fungal inactivation in rice grains. The optimal conditions of dielectric heating are suitable for fungal inactivation in rice industries. These results indicate that the proposed dielectric heating system is useful for inactivation of Aspergillus species.

Keywords: dielectric heating; fungal inactivation; electric field intensity; energy consumption

1. Introduction

Rice (Oryza sativa L.) is one of the most important grains that feed approximately 75% of the world’s population. It is a staple food of Asian countries, including China, India, Indonesia, Bangladesh, Vietnam, Philippines, Thailand, etc. [1,2]. However, the quality of rice grains can be affected by contaminated fungal contamination Aspergillus species [3,4]. Aspergillus species contamination levels in brown rice were discovered to be 10^3 CFU/g [5]. Rice contamination with fungal species during harvest and storage is of poor quality and low economic value. Moreover, it can harm human and animal health [6]. Some fungal species (mainly Aspergillus flavus and Aspergillus parasiticus) produce aflatoxin, which are fungal secondary metabolites that contaminate dietary staples worldwide, including maize, rice, and groundnuts [7]. Dietary exposure to aflatoxin is a public health concern due to its acute and chronic carcinogenic effects [8]. Therefore, decontamination of fungi in rice grains before storage or distribution is very important.

Techniques for fungal decontamination have been reported, including chemical, biological, and physical methods [9]. Various chemicals, such as ozone (O_3), have the potential to inhibit fungal growth and disrupt mycelial development through oxidation reactions. However, the use of ozone limitations in industrial applications, such as the high-cost requirement for installation and operation [10,11]. Biological methods, such as temperature, humidity, and other environmental parameters, do not kill or inhibit fungi, especially fungal spores [12]. Interestingly, there are physical mechanisms, such as radio frequency
heating (RFH) and microwave heating (MWH). The methods can potentially damage fungal cells by heat generation [13–15], which has no toxic residues and no effect on human health. In addition, the rice industry has used RFH and MWH treatments [16–18].

RFH and MWH have been used for controlling various fungi in food products. For example, RF treatment used to control *Aspergillus flavus* in oilseed, wheat, and corn [19,20] and *Penicillium crustosum* in chestnuts [21]. The control of *Aspergillus flavus* and *Aspergillus parasiticus* in brown rice, barley, and corn [5,22], *Penicillium* spp., and *Aspergillus* spp. in Brazil nut seed shell and kernel has been reported using MW [23]. At the present study, dielectric heating is one of the heating techniques, including RF and MW energy. This technique can convert electrical energies into thermal energy inside the dielectric material. Depending on the electric field frequency, different heat level is generated in the dielectric material [24–28]. In previous studies, the application of dielectric heating is a selective heating technique for insect control in rice [29–37]. The method involves heating the insects to a lethal temperature (60 °C) with exposure time (30 min) and avoiding the heat generated in the rice based on the different dielectric properties [33,38]. The dielectric loss is the ability to dissipate energy in an applied electric field [31,33]. The measured dielectric loss is considered to find out the optimal frequency ranges, which leads to efficient heating system.

This is novel research that aims to optimize the best condition of dielectric heating for fungal control in rice products. The comparative effect of these dielectric heating frequency ranges on fungal growth had not yet been conducted. In addition, the effect of electric field intensity on the heating system is also examined in this study. The information about dielectric properties is an important key for developing dielectric heating industrial equipment in the future.

2. Materials and Methods

2.1. Rice Samples Preparation and Fungal Strain

Thai Hom Mali brown rice (*Oryza sativa* L. cv. KDML105) was purchased from the local market in Nakhon Ratchasima city, Thailand. The rice was taken out and allowed to equilibrate to room temperature (25 ± 2 °C) overnight. The moisture content of the rice sample was measured using a digital grain moisture meter (Handheld SMART SENSOR AR991, Hebei, China). Five Kg of dehulled polished rice samples was grounded to a fine powder using a grinding machine (Huangcheng HC-200, Shanghai, China), as shown in Figure 1a.

![Figure 1a](image1a.png)

![Figure 1b](image1b.png)

**Figure 1.** The sample of dielectric properties measurement: (a) Rice powder sample; (b) *Aspergillus* sp. BP17.
The fungal strain BP17 was identified as Aspergillus sp. and procured from the culture collection of AJTR microbiology laboratory, School of Preclinical Sciences, Institute of Science, Suranaree University of Technology (Nakhon Ratchasima city, Thailand) with deposited number as BP17. The strain was cultured on Potato Dextrose Agar (PDA; Himedia, Maharashtra, India) and incubated at 30 °C for one week, as shown in Figure 1b.

2.2. Measurement of Dielectric Properties of Fine Rice Powder and Aspergillus sp. BP17

The fungal colony on PDA and 60 g of fine rice powder in a polystyrene Petri dish (diameter 100 mm and height 15 mm) were measured dielectric properties using a VNA (Agilent Technologies, Inc., Santa Clara, CA, USA) with an open-ended coaxial probe and a computer [39,40]. The probe and the network analyzer were contacted using a highly flexible coaxial. The network analyzer measurement based on software (Keysight Materials Measurement Suit; Keysight Technologies Inc., Santa Rosa, CA, USA) was set from 40 to 8500 MHz and at 1001 discrete frequencies to measure the dielectric properties. The measurement set-up is shown in Figure 2.

![Measurement equipment](image)


The measurement system was turned on and kept in a standby mode at room temperature for approximately 60 min. Then, the network analyzer and the open-end coaxial probe were calibrated at room temperature using air, short, and deionized water as the standard [41]. After that, the calibrated probe was dried and cleaned using 70% alcohol.

For dielectric properties measurement, the samples (fungal colony on PDA and 60 g of fine rice powder) were placed on the laboratory jack and the sample surface was touched with the probe (Figure S1 in Supplementary Material). Then, the dielectric property values of each sample were measured with three replications, and the values were recorded and analyzed for optimal frequency ranges in dielectric heating experiments.

2.3. Experimental Desing for Dielectric Heating System

2.3.1. Dielectric Heating System Setting

The dielectric heating system (9 kW, 40.68 MHz) was used in this study. This system mainly consisted of two parallel electrode plates and a frequency oscillator as shown in Figure 3a. The upper and bottom electrode plate size is 52 cm × 52 cm. The position of the upper electrode can be adjusted to achieve a different electric field intensity while the bottom electrode plate is fixed for placing the samples, the central position of the bottom electrode provides a better heating rate with a more uniform temperature distribution [42]. The sterile Petri dish containing the rice sample was placed at the center of the bottom electrode, as shown in Figure 3b.
For dielectric heating, the power conversion in a material depends on the operating frequency, dielectric loss factor and the electric field density inside the material. The power dissipated per unit volume in the dielectric material can be expressed [43] as Equation (1):

$$ P = f E^2 \varepsilon'' 55.63 \times 10^{-12} $$

where $P$ is the power dissipated per unit volume (W/m$^3$), $f$ is the frequency (Hz), $E$ is the rms electric field intensity (V/m) and $\varepsilon''$ is dielectric loss factor of the material being exposed to the alternating electric fields.

2.3.2. Determining the Optimal ISM Frequency Bands

Specific frequency range of RF and MW were studied at 40 to 8500 MHz based on the industrial, scientific, and medical (ISM) frequency [44]. Then, the dielectric constant ($\varepsilon'$) and loss ($\varepsilon''$) at 40.68 MHz of RF, and 915 and 2450 MHz of MW were measured and analyzed. The optimal frequency was calculated using the ratio between dielectric properties of *Aspergillus* sp. BP17 and rice samples, and the highest of the ratio was chosen as optimal ISM frequency (MHz).

2.3.3. The Study of Relative Electric Field Intensity and Temperature

The dielectric heating system was set the optimized ISM frequency band as 40.68 MHz. The electric field intensity was studied and calculated according to Equation (2):

$$ E = \frac{V}{\Delta d} $$

where $E$ is the electric field intensity (V/m), $V$ is the voltage during the heating process (V), $\Delta d$ is the distance between electrodes (m).

In total, 75 dehulled polished rice samples (60 g with 8.33% moisture content) were prepared in sterile glass Petri dish and then placed at the central position of the bottom electrode under the dielectric heating system at 40.68 MHz frequency. The temperature was monitored and recorded using the infrared thermography camera (U5857A, Keysight...
Technologies Inc., Santa Rosa, CA, USA). At different five electric field intensities (150, 190, 225, 300, and 450 kV/m), heating time \( t \) was recorded three replicates at each five temperatures \( T \) including 70, 80, 90, 100, and 110 °C and calculated for heating rate \( \mathcal{C}/\text{min} \) according to Equation (3):

\[
\text{Heating rate} = \frac{\Delta T}{\Delta t}
\]

where \( T \) is the temperature in sample (°C) and \( t \) is the heating time (min).

The energy consumption \( W \) was calculated according to Equation (4):

\[
W = \frac{VI\Delta t}{m}
\]

where \( W \) is the energy consumption (kWh g\(^{-1}\)), \( V \) is the voltage during the heating process (V), \( I \) is the current during the heating process (A), \( t \) is the heating time (hour), and \( m \) is the sample mass (gram).

2.3.4. Effect of Dielectric Heating on Fungal Growth

The treated seventy-five rice samples in a sterile glass Petri dish (Figure 4a) were treated at different electric field intensities (150, 190, 225, 300, and 450 kV/m) and temperatures (70, 80, 90, 100, and 110 °C) using dielectric heating system. The non-treated rice sample was used as control with three replications. The rice samples were kept in a sterile glass Petri dish during the heating process using aseptic techniques [45]. After that 20 grains of treated and non-treated (control) rice samples were randomly selected and placed on a sterile PDA using aseptic techniques under a laminar airflow cabinet (ScanLaf/Mars 1500, Labogene Aps, Lynge, Denmark) (Figure 4b). Then, the plates were incubated at room temperature for five days. The fungal mycelium was observed using a stereo microscope (Stemi 305, Carl Zeiss, Oberkochen, Germany) and compared with control groups that were not exposed to the electric field. Fungal colonies on rice seeds were counted and fungal inactivation was calculated according to Equation (5):

\[
\text{fungal inactivation (\%)} = \left( 1 - \frac{N_P}{N_T} \right) \times 100\%
\]

where \( N_P \) is the number of positive rice grain, and \( N_T \) is the number of total rice grain.

![Sample images](image-url)  
**Figure 4.** Samples for investigating fungal inactivation: (a) rice sample; (b) rice grains on PDA before incubation.
2.4. Statistical Analysis

Each experiment was carried out in triplicate and data were statistically analyzed using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to determine the statistical differences between the sample means, with the level of significance set at 5% probability. Multiple comparisons of the means were conducted using a factorial experiment test. All data were expressed as mean ± SD.

3. Results and Discussion

3.1. Optimization of Frequency Band under Dielectric Heating System

The dielectric properties [dielectric constant (ε′) and loss (ε″)] of Aspergillus sp. BP17 and rice powder samples were investigated at frequency ranges as 40 to 8500 MHz as shown in Figure 5. The result showed that dielectric constants of both Aspergillus sp. BP17 and rice powder were decreased when frequency increased. In accordance with Cheng et al. (2017) reported that the electric constant is generally decreases when frequency increases for all types of rice due to the presence of polar molecules such as glucose and fructose [40]. At low frequencies (<200 MHz), the dielectric constants of Aspergillus sp. BP17 rapidly decreased from 31.5 to 21.8 at frequencies between 40 to 200 MHz (Figure 5a, upper line). A previous study reported that the dielectric constants of fungi were higher than 4.0 at frequencies lower than 200 MHz including Botrytis cinerea, Alternaria solani, Ceratocystis radicicola, Rhizoctonia solani, Lasiodiplodia theobromae, Fusarium oxysporum and F. solani [39]. However, the dielectric constants of rice powder slowly reduced from 5.06 to 4.63 at frequencies between 40 to 200 MHz (Figure 5a, bottom line). In comparison between fungi and rice samples, Aspergillus sp. BP17 showed higher dielectric constant than the rice sample due to it being associated with cell membrane polarization [46].

![Figure 5. Dielectric properties of Aspergillus sp. BP17 and rice powder: (a) Dielectric constant (ε′) and (b) Dielectric loss (ε″).](image-url)

The dielectric loss of Aspergillus sp. BP17 rapidly decreased from 48.92 to 13.13 at frequencies between 40 to 200 MHz (Figure 5b, upper line). After that, the values were slowly increased when frequencies were increased between 2000 and 8500 MHz. In contrast, the dielectric loss of rice was 0.26 and 0.86 at frequency of 40–200 and 8500 MHz, respectively (Figure 5b, bottom line). Aspergillus sp. BP17 and rice powder showed a different dielectric loss because the change of dielectric loss was caused by the relative effects of ionic conductivity and free water relaxation [40,41,47]. Moreover, the dielectric loss behavior of Aspergillus sp. BP17 was similar to the results reported in edible fungi [47].
and the dielectric loss behavior of rice in this study was also similar to Jasmine rice 105, Hompathum rice, Phitsanulok rice, Chinart rice, and Gorkor 43 rice [48].

According to the US Federal Communication Commission (FCC), it has been reported that the optimal specific frequencies for ISM uses 13.56, 27.12, and 40.68 MHz of RF and 915 and 2,450 MHz of MW [44]. So, three ISM frequency bands, including 40.68, 915, and 2450 MHz, were selected and calculated the ratio of dielectric constant and loss in fungal and rice powder samples (Table 1). The result showed that optimal frequency was 40.68 MHz based on the highest of ratio (194.9 folds) between dielectric loss of Aspergillus sp. BP17 and rice powder, which is the same result with Figure 5b. Therefore, at a frequency with the high difference of dielectric loss between fungi and rice can be applied for fungal inactivation using dielectric heating applications based on the “thermal runaway” phenomenon as described [33]. Therefore, the frequency of 40.68 MHz was selected for fungal control using dielectric heating system in the next experiment.

### Table 1. Dielectric properties of Aspergillus sp. BP17 and rice powder.

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>Aspergillus sp. BP17 (A)</th>
<th>Rice powder (B)</th>
<th>Ratio A:B</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.68</td>
<td>31.574 ± 0.333</td>
<td>4.754 ± 0.123</td>
<td>6.641</td>
</tr>
<tr>
<td>915</td>
<td>18.356 ± 0.449</td>
<td>3.774 ± 0.044</td>
<td>4.863</td>
</tr>
<tr>
<td>2450</td>
<td>16.794 ± 0.166</td>
<td>3.343 ± 0.064</td>
<td>5.024</td>
</tr>
<tr>
<td>40.68</td>
<td>48.92 ± 0.466</td>
<td>0.251 ± 0.036</td>
<td>194.900</td>
</tr>
<tr>
<td>915</td>
<td>5.042 ± 0.117</td>
<td>0.551 ± 0.022</td>
<td>9.151</td>
</tr>
<tr>
<td>2450</td>
<td>4.412 ± 0.106</td>
<td>0.582 ± 0.019</td>
<td>7.580</td>
</tr>
</tbody>
</table>

3.2. Study of Relative Electric Field Intensity and Temperature on Heating System

The effect of relative electric field intensities and temperatures (heating time-temperature histories and energy consumption-temperature histories) was investigated at the selected frequency of 40.68 MHz (Figure 6). The result showed that the heating time-temperature histories and energy consumption-temperature histories were nonlinear. The graph also indicates that the heating time and energy consumption decreased when electric field intensities increased. The optimal electric field intensity was 450 kV/m based on the highest ratio (26.26 folds at 90 °C) between the heating time comparison of 450 kV/m and 150 kV/m for each temperature (35 min, and 47.25 °C/min). The differences in the heating time are because of various heating mechanisms [49]. It has been reported that dielectric materials convert electric energy to heat when placed into an electromagnetic field in the range of radio and microwave frequencies [43,50]. Similar to the earlier work, heating rate and heating time changed with electrode gaps [51–53]. Moreover, the optimal electric field intensity was 450 kV/m based on the highest of ratio (26.26 folds at 90 °C) between the energy consumption comparison of 450 kV/m and 150 kV/m for each temperature (0.33 kWh/g). This result showed that the increased electric field intensity allowed less energy in the dielectric heating system. Similar to the earlier work, energy comparison changed with electrode gaps (1.98 folds at 80 °C) [49].

3.3. Effect of Dielectric Heating Condition on Fungal Growth

The rice samples were treated by the heating system at a selected frequency and electric field intensity of 40.68 MHz with 150, 190, 225, 300, and 450 kV/m, respectively. The fungal inactivation on the rice sample was obtained by using temperature with electric field intensity (Figure 7). As seen in Figure 7, the synergies of temperatures and electric field intensities can be divided into nine groups (a–i, which are from the highest to lowest group of the fungal inactivation). The result showed that the fungal inactivation of 100% was obtained at the electric field intensity value $\geq 225$ kV/m at the lowest temperature of 90 °C. Based on statistical analysis, the fungal inactivation in rice grains was significant ($p \leq 0.05$) by the effects of the combination of temperature and electric field intensity. A previous study reported that the effect of both the thermal and non-thermal was caused by the
pasteurization mechanism on micro-organisms at the cellular level [54]. Here, the fungal inactivation of 100% was obtained using 190 kV/m at 100 and 110 °C, 225 kV/m at 90, 100 and 110 °C, 300 kV/m at 90, 100 and 110 °C, 450 kV/m at 90, 100 and 110 °C (Figure 7). The effect of different temperatures on fungal inhibition has been reported including Aspergillus sp. in canned strawberries (94 °C) [55] and grape juice (85 °C) [56], Aspergillus niger (90 °C) and Aspergillus flavus in oil seed (90 °C) [19], Aspergillus flavus in Khao Dawk Mali 105 (90 °C) [57], and the other Aspergillus species with a neosartorya-morph (80–90 °C) [58].

Figure 6. The relative electric field intensity and temperature in the heating system.

Figure 7. The relative electric field intensity and temperature on fungal growth. Average ± standard deviation from three replicate experiments. Different letters in the same column are considered significantly different according to Duncan’s multiple comparison test (p ≤ 0.05).
To visualize the effect of dielectric heating on fungal growth based on different electric intensities, six samples including one non-treated sample (control) and five treated samples (150, 190, 225, 300, and 450 kV/m) were selected to show fungal inactivation. Figure 8 shows the fungal growth on PDA after rice samples were treated with different electric intensities at 90 °C. Fungal mycelium was grown from rice grains in the control agar plate. On the contrary, fungal growth from rice grains was deceased based on 150 kV/m and 190 kV/m, and the hyphae were not germinated from rice grains that were treated with electric field intensity value $\geq 225$ kV/m at 90 °C. This indicates that the optimal conditions of dielectric heating (combination of 225 kV/m and 90 °C) are powerful for fungal inactivation in rice grains by dielectric heating. Cain reported that the electric field (194 kV/m) has a non-thermal effect on the cellular membrane (in the air of electrode gaps) [59]. Use of both thermal and non-thermal mechanisms was reported in Aspergillus fumigatus and Neosartorya fischeri in apple juice (83 and 92 °C with 20 V/cm, 83 °C with 27 V/cm, and 90 °C with 9 V/cm) [60,61] and Aspergillus niger in tomato (89 °C with 36 V/cm) [62].

Control 150 kV/m 190 kV/m 225 kV/m 300 kV/m 450 kV/m

Figure 8. The fungal growth on Petri dish after treated with different electric intensity at 90 °C.

The results indicate that the contaminated fungi in rice grain products can be eliminated using the dielectric heating machine. The system can generate heat on fungal cells faster than the surrounding media/environments. As a result, the rapid oscillation of the fungal cell leads to elastic limit, rupture, and cell death [63].

This study suggested that dielectric heating is an effective method for fungal inactivation in rice grains. Additionally, the optimal conditions of dielectric heating are suitable for inactivation of contaminated fungi in rice industries.
4. Conclusions

In the present study, the dielectric properties between Aspergillus sp. BP17 and rice were studied, and the optimal frequency of 40.68 MHz was selected for fungal inactivation using the dielectric heating system. The optimization of dielectric heating for fungal inactivation was evaluated by the relationship between electric field intensity and temperature. The results showed that dielectric heating treatment killed or inhibited fungi on rice grains. The best condition (energy consumption of 0.33 kWh/g and fungal inactivation of 100%) was obtained at 450 kV/m and 90 °C. This study provides knowledge of dielectric heating for developing the heating system and equipment for rice products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122010478/s1, Figure S1: Measuring dielectric properties of sample: (a) Rice powder sample; (b) Aspergillus sp. BP17.


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