

# Edible Thai rice film incorporated with ginger extract by microwave extraction: optimization of bioactive compounds and functional properties for antimicrobials

**Niramon Utama-ang**

Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100th

**Sirinapa Sida**

Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100th

**Phenphichar Wanachantararak**

Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200

**Arthitaya Kawee-ai** (✉ [kaweeai@gmail.com](mailto:kaweeai@gmail.com))

Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100th

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## Research Article

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4 Niramon Utama-ang<sup>1,2,3</sup>, Sirinapa Sida<sup>1</sup>, Phenphichar Wanachantararak<sup>4</sup>, Arthitaya Kawee-ai<sup>1,\*</sup>

5

6 <sup>1</sup> Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai  
7 University, Chiang Mai, 50100, Thailand

8 <sup>2</sup> Cluster of High Value Product from Thai Rice and Plants for Health, Chiang Mai University,  
9 Chiang Mai, 50100, Thailand

10 <sup>3</sup> Research Center for Development of Local Lanna Rice and Rice Product, Chiang Mai  
11 University, Chiang Mai 50200, Thailand

12 <sup>4</sup> Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand

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14 **Running title:** Applying microwave ginger extract on rice film...

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16 \* Corresponding author: Dr. Arthitaya Kawee-ai

17 Tel.: +66 979783688, Fax: -

18 E-mail: kaweeai@gmail.com

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21 **Abstract**

22 This study aimed to integrate microwave-assisted extraction (MAE) on the dry ginger extract  
23 and to develop rice-based edible film incorporated with ginger extract. An efficient MAE was  
24 developed to extract the dried ginger using a 3<sup>2</sup> full factorial design. The optimal condition was  
25 a microwave power of 400W and an extraction time of 1 min. The extraction time was a  
26 significantly effective factor than microwave power, whilst power was not a significant factor  
27 for yield, 6-gingerol, 6-shogaol, and paradol. A crude extract of dried-ginger has antimicrobial  
28 activity against *S. mutans* DMST 18777 with minimum inhibitory concentration (MIC) and  
29 minimum bactericidal concentration (MBC) of 0.49 mg/mL and 31.25 mg/mL, respectively.  
30 The rice-based edible film incorporated with 32 mg/mL of ginger extract against *S. mutans*  
31 DMST 18777 with a mean zone of inhibition of 12.69±0.07 mm. The functional property of  
32 rice film was remarkably better than the original. Significant increases in TPC, antioxidant and  
33 bioactive compounds were associated with increase ginger extract contents in rice film. The  
34 main phenolic compounds including 6- gingerol 6-shogaol, paradol, and zingerone, and  
35 essential oils including  $\alpha$ -curcumene,  $\alpha$ -zingiberene,  $\gamma$ -muurolene,  $\alpha$ -farnesene,  $\beta$ -bisabolene,  
36 and  $\beta$ -sesquiphellandrene were found in rice film strip fortified with crude ginger extract.

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38 **Keywords:** microwave-assisted extraction; rice-based edible film; antimicrobial activity;  
39 ginger (*Zingiber officinale*); bioactive compound

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## Introduction

Recently, natural additives from plant extracts incorporation in edible films or coating have gained increasing attention among researchers. Natural additives have been applied to improve texture, rheology, and functional properties such as antioxidant, antimicrobial, anti-browning, of the edible films <sup>1</sup>. Rice (*Oryza sativa* L.) is one of the main foods for almost half of the global population. Rice flour is widely used as food hydrocolloids due to its inexpensive, convenient, biodegradable, and easy processability <sup>2</sup>. Furthermore, rice flour can be formulated with other components to improve the physical, chemical, sensory, and nutritional properties of the product <sup>3</sup>. The use of rice flour to produce films or coating is generally transparent, odorless, colorless, and tasteless <sup>2</sup>.

*Zingiber officinale* Roscoe (ginger) as a member of the Zingiberaceae family, is commonly used as a spice in food and as a traditional medicine in Asian countries. Many bioactive compounds of ginger such as phenolic compounds (gingerols, shogerols, and paradols), terpenes (zingiberene,  $\beta$ -bisabolene, and  $\alpha$ -curcumene), polysaccharides, lipids, and organic compounds have processed multiple biological activities nowadays, for example, antioxidant activity, anticancer, anti-diabetic, anti-inflammatory, antimicrobial activity, cardiovascular protective <sup>4</sup>. However, the use of a conventional method to extract the bioactive compounds required a long extraction time and it affects the quality of a final product by losing some of the volatile compounds which leads to low extraction efficiency <sup>5</sup>. The microwave-assisted technique has been widely employed to extract phenolic compounds from plants due to its small equipment size, simplicity, and rapidness. The efficiency of the MAE was found to be two times higher than that of the conventional method <sup>6,7</sup>.

In the previous study, 9-months of dried ginger at 60 °C for 308 min presented the highest TPC of 12.21  $\mu\text{mol}$  tannic acid/g and 6-gingerol of 12.57 mg/g <sup>8</sup>. Furthermore, the nonvolatile compounds including gingerols, shogaols, paradols, and zingerone in ginger exhibit

antioxidant activity, which shogaols, paradols, and zingerone presented in a high content while drying and roasting<sup>9</sup>. Thus, the first objective was to investigate the effect of MAE condition, microwave power and extraction time, on yield, antioxidant activity, and bioactive compounds of dry ginger. The second objective was to integrate crude ginger extract into rice film and to determine antimicrobial activity, antioxidant activity, and bioactive compounds.

## **Results**

### **Effect of microwave power and extraction time on ginger extraction yield**

To define the effect of MAE, the microwave power (400, 600, and 800 W) and time (1, 3, and 5 min) were set to determine the extraction yield, TPC, DPPH, ABTS, FRAP, 6-gingerol, 6-shogaol, paradol, and zingerone from dry ginger (Table 1). There was a significantly improve the extraction yields with an increase of microwave power from 400 to 800 W, however, the yields were decreased with further increase extraction time ( $p \leq 0.05$ ). The yield of crude extract was approximately  $7.31 \pm 1.01$ - $10.70 \pm 1.31\%$ . The regression equation of the yield was fitted with a coefficient of determination ( $R^2$ ) of 0.7044 (Table 2). Extraction yield showed a positive correlation with microwave power and time, however, the combination of microwave power and extraction time showed a negative effect, which was correlated with the contour plot (Figure 1 A). The yield of crude extract initially increased with the increase in microwave power and extraction time. However, the increase in microwave power  $>600$ W resulted in a decrease in the extraction yield.

### **Effect of microwave power and extraction time on TPC and antioxidant activity**

Conversely, TPC and antioxidant activities measured by DPPH, ABTS, and FRAP methods significantly decreased with the increase of both power and time ( $p \leq 0.05$ , Table 1). The highest value of TPC and DPPH activity was found to be  $200.84 \pm 4.54$  mg GAE/g and  $91.87 \pm 0.42\%$ , respectively, at 400 W and 1 min of MAE condition. Whilst, ABTS and FRAP values presented at  $120.86 \pm 2.33$  mg/g and  $377.86 \pm 4.29$  mg/g when 800 W of microwave power and 5 min was

used. The summary of the analysis of variance (ANOVA) representing results indicated that TPC, ABTS, and FRAP values were reliable and significant ( $p < 0.05$ ) with  $R^2$  values of 0.9758, 0.9267, and 0.09005, respectively (Table 2). Microwave power and extraction time were found to be non-significant ( $p > 0.05$ ) on these responses, which means that the increase of microwave power and extraction time was decreased the TPC, ABTS, and FRAP values. However, the interaction of microwave power and extraction time was represented the positive signs on the observed value. Consistent with the ANOVA results, MAE presented a negative effect for TPC (Fig. 1 B), ABTS (Fig. 1 C), and FRAP (Fig. 1 D) with a significant decrease in their values when a high microwave power ( $>500\text{W}$ ) and long extraction time ( $>2$  min) was applied.

### **Effect of microwave power and extraction time on bioactive compounds**

The highest yield of 6-gingerol was obtained either with low microwave power and short extraction time (400, 1 min) and/or high microwave power and long extraction time (800 W, 5 min). The highest content of paradol could be produced when microwave power is in a range of 400-600 W. While, the highest yield of 6-shogaol and zingerone could be achieved when microwave power is 800 W and with the long extraction time of 5 min. 6-Gingerol and paradol were showed the highest value of  $73.41 \pm 1.33$  mg/g and  $23.51 \pm 0.87$  mg/g at low power (400 W, 1 min). Whilst, 6-shogaol, and zingerone represented the highest value at high power and longer extraction time (800 W, 5 min) with the values of  $16.04 \pm 1.86$  mg/g and  $5.79 \pm 0.06$  mg/g, respectively (Table 1). However, the use of MAE on extracted bioactive compounds from ginger could be generated the model for only 6-gingerol ( $p < 0.05$ , Table 2). High values for  $R^2$  were achieved as 0.9267 and microwave power and extraction time were found to be non-significant ( $p > 0.05$ ). Consistent with the ANOVA and contour plots of TPC, ABTS, and FRAP, MAE represented the negative effect for 6-gingerol with a significant decrease in their values when a high microwave power ( $>500\text{W}$ ) and long extraction time ( $>2$  min) was used (Fig. 1 E).

### **Optimization and validation of MAE condition**

The contour response surfaces were plotted to study the interactions between the factors on the significant responses to determine the optimum levels of each factor required to obtain maximum values of the yield, TPC, ABTS, FRAP, and 6-gingerol (Figure 2). Based on the analysis and calculation, the validation experiment was conducted as microwave power of 400 W and 1 min of extraction time. The highest yield of crude ginger ( $7.66\pm 0.66\%$ ) was closed to the predicted value of 7.61%. TPC and antioxidant activities including ABTS and FRAP values were  $198.24\pm 0.74$  mg GAE/g,  $106.42\pm 3.13$  mgTrolox/g, and  $304.62\pm 5.49$  mgTrolox/g, respectively (Table 3). The highest yield of 6-gingerol was obtained as  $71.57\pm 3.60$  mg/g. The percentage of approximated error between predicted and experimental values was in the range of 0.07-4.38%, which not above 10% of the approximated error. This indicated that the results of the validation were acceptable and consistent with the predicted values.

### **The application of crude ginger extract in rice-based edible film**

#### **Antimicrobial activity of rice-based edible film**

The disc diffusion analysis results revealed that the crude extract of ginger (63-500 mg/mL) showed no significant difference ( $p < 0.05$ ) on antimicrobial activity against *S. mutans* DMST 18777 with an average inhibition zone of  $9.50\pm 0.71$ - $11.00\pm 1.41$  mm (Table 4). The MIC and MBC of the crude extract of ginger against *S. mutans* DMST 18777 were 0.49 mg/mL and 31.25 mg/mL, respectively. Thus, the highest concentration of crude ginger extract that was applied into the rice-based edible film was 32 mg/mL (3.2%, w/v). Therefore, inhibition zone diameters yield by rice-based edible film disks with various concentrations (0, 4, 8, 16, and 32 mg/mL or 0.4, 0.8, 1.6, and 3.2%, w/v) of crude ginger extract against *S. mutans* DMST 18777 are presented in Table 3. No inhibition zone against *S. mutans* DMST 18777 was observed for rice film without the incorporation of crude ginger extract, which indicating that rice film alone did not affect antimicrobial activity. Furthermore, the incorporation of crude ginger extracts

less than 16 mg/mL in the rice film not enough to inhibit the growth of *S. mutans* DMST 18777. The efficiency concentration was 3.2 % (w/v) with an inhibition zone of 12.69±0.07 mm.

### **The change of TPC, antioxidant activity, bioactive compounds, and volatile compounds of rice film**

The TPC, antioxidant activity, and bioactive compounds (6-gingerol, 6-shogaol, paradol, and zingerone) of the edible rice films increased gradually throughout the increase of crude ginger extract contents (Table 5). Paradol and zingerone were absented from rice film with 4.0 mg/mL of ginger extract. The main bioactive compounds that were presented in the rice film were 6-gingerol followed by 6-shogaol, paradol, and zingerone, respectively. Meanwhile, the principal components of the volatile compounds of crude ginger including  $\alpha$ -curcumene,  $\alpha$ -zingiberene,  $\gamma$ -muurolene,  $\alpha$ -farnesene,  $\beta$ -bisabolene, and  $\beta$ -sesquiphellandrene showed no significant difference percentage ( $p < 0.05$ ) in the rice film. The main compound found in the rice film fortify with crude ginger extract was followed by  $\alpha$ -zingiberene >  $\alpha$ -curcumene  $\geq$   $\beta$ -sesquiphellandrene >  $\alpha$ -farnesene >  $\gamma$ -muurolene  $\geq$   $\beta$ -bisabolene. Whist, rice film without ginger extract showed no TPC, antioxidant activity, bioactive compounds, and volatile compounds.

### **Discussions**

Ginger is common and widely used as a spice and herbal medicine for a long time. The bioactive compounds such as 6-gingerol and 6-shogaol were accounted for several bioactivities including antioxidant, anticancer, antimicrobial, and anti-inflammatory<sup>10</sup>. Ginger is known to harden the teeth because of indirect mineralization properties, thus, ginger was validated for oral care<sup>11</sup>. While rice is a staple food that is consumed by half of the global population. Furthermore, there is no information about the effect of the combination of crude ginger extract on rice film properties. In this study, we optimized the MAE condition in order to increase the

yield, antioxidant activity, and bioactive compounds of dried ginger, and then combined the crude extract into an oral rice film strip.

Generally, the increase of microwave power could be increased the extraction yield with a shorter extraction time <sup>12</sup>. The change of antioxidant activities might be due to the generation of free radicals such as H<sup>+</sup>, OH<sup>-</sup>, and electrons through microwave radiation <sup>4</sup>. The highest yield of 6-gingerol was obtained either with low microwave power and short extraction time (400, 1 min) and/or high microwave power and long extraction time (800 W, 5 min), which agreed on the results of Teng *et al.* <sup>5</sup>. Under high temperatures and/or high microwave power, 6-gingerol dehydrated water (H<sub>2</sub>O) from its structure and converted to 6-shogaol <sup>4</sup>. If the reduction of (CH<sub>2</sub>)<sub>2</sub> occurred, 6-shogaol will be transformed into paradol. In another case, microwave power promoted retro-aldol reaction of 6-gingerol and proposed to generate zingerone constituents with an aldehyde to deliver the products (Figure 3). The competition of these reactions can be further demonstrated by the synthesis of the 6-shogaol, paradol, and zingerone constituent's yields. The content of 6-shogaol and zingerone was gradually increased under high microwave power (>600 W) and long extraction time (>3 min). This indicated that 6-shogaol and zingerone were produced during high temperatures, high microwave power, and also by thermal degradation of gingerol <sup>9</sup>. 6-Shogaol and zingerone increased with increasing the microwave power and extraction time, which results in increased ABTS and FRAP values. This phenomenon could be explained that 6-gingerol was dehydrated and generated H<sup>+</sup> and OH<sup>-</sup> radical at high temperatures or high microwave power results in produced 6-shogaol, paradol, zingerone, and its derivatives <sup>5,6</sup>.

*S. mutans* is found in the oral cavity and formed a dental plaque to prevent the permission of antimicrobial agents <sup>11</sup>. The oral film strip is considered one of the most convenient routes for administration due to cost efficiency and ease of administration. In this study, we observed the inhibitory activity of crude ginger extract and found that the MIC and

MBC of the crude ginger extract against *S. mutans* DMST 18777 were 0.49 mg/mL and 31.25 mg/mL, respectively, with the inhibition zone approximately 11.0 mm. These results agree with that reported by Mathai *et al.* <sup>13</sup>, where fresh ginger extract against *S. mutans* MTCC 497 with the inhibition zone of 11.72±0.62 mm. Furthermore, many studies also showed the inhibition zone of fresh ginger extract against *S. mutans* approximately 6-18 mm <sup>14-16</sup>. The incorporation of crude ginger extracts less than 16 mg/mL in the rice film not enough to inhibit the growth of *S. mutans* DMST 18777. This might be due to the immobilization of rice molecules within the film and a high number of bacteria ( $2.76 \times 10^6$  CFU/mL or 6.44 log CFU/mL) that exceed inhibition activity <sup>17</sup>. The antimicrobial activity of crude ginger against *S. mutans* was 5 logs CFU/mL <sup>17</sup>. The efficiency concentration was 32 mg/mL (3.2% w/v) with the inhibition zone of 12.69±0.07 mm. It can be concluded that the produced rice-based edible film with 32 mg/mL of ginger extract has the potential to be considered for anti-caries rice film. In contrast, the starch edible film with ginger essential oils (1-3% v/w) inhibited the growth of *Escherichia coli* with 1.00-9.73 mm of inhibition zone <sup>18</sup>. It seems that gram-negative bacteria (*E. coli*) were more resistant to lipophilic compounds as compared with gram-negative bacteria (*S. mutans*), which occupied a single peptidoglycan layer structure <sup>19</sup>.

The presence of 6-gingerol and 6-shogaol in the rice film strip could be imparted a pungent and aromatic taste to the film. 6-Gingerol, 6-shogaol, paradol, and zingerone can inhibit reactive oxygen species and maintain their antioxidant properties <sup>9</sup>. Thus, the antioxidant activity of rice film might be related to the presence of TPC and bioactive compounds. The essential oil of crude ginger was found to be 44 volatile compounds <sup>8</sup>. In this study, the main essential oil in the rice film strip was  $\alpha$ -curcumene,  $\alpha$ -zingiberene,  $\gamma$ -muurolene,  $\alpha$ -farnesene,  $\beta$ -bisabolene, and  $\beta$ -sesquiphellandrene, as identified by GC/MS. These compounds are sesquiterpenes. The monoterpenes and sesquiterpenes are the main classes of volatile compounds of ginger, which  $\alpha$ -zingiberene, camphene,  $\alpha$ -farnesene, and  $\beta$ -

sesquiphellandrene are attributed to the antioxidant activity <sup>20</sup>. In this study,  $\alpha$ -zingiberene (27.06 $\pm$ 4.29 - 34.60 $\pm$ 6.69%) was the main compounds of the essential oil in the rice film strip, followed by  $\alpha$ -curcumene (13.29 $\pm$ 2.09 - 16.86 $\pm$ 2.93%),  $\beta$ -sesquiphellandrene (13.13 $\pm$ 4.58 - 15.38 $\pm$  2.41%),  $\alpha$ -farnesene (10.36 $\pm$  1.63 - 12.77 $\pm$  2.01%),  $\beta$ -bisabolene (6.27 $\pm$  2.24 - 8.69 $\pm$ 1.06%), and  $\gamma$ -muurolene (6.30 $\pm$ 0.37 - 7.64 $\pm$ 1.21%). Wang et al. <sup>21</sup> showed that  $\alpha$ -zingiberene was the main compounds of the essential oil found in ginger which range from 17.4-25.4%, followed by  $\alpha$ -curcumene (14.1-16.4%),  $\beta$ -bisabolene (9.9-12.5%), and  $\beta$ -sesquiphellandrene (9.7-13.4%), which was consistent with this study.

## Conclusions

MAE was successfully used to extract TPC, 6-gingerol, 6-shogaol, paradol, and zingerone from dried ginger, and increased antioxidant efficiency within shorten extraction time by 3<sup>2</sup> full factorial design. The optimal condition was microwave power of 400W and extraction time of 1 min and showed the responses which were close to the predicted responses. For antimicrobial activity, the crude extract of dried-ginger against *S. mutans* DMST 18777 with MIC and MBC values of 0.49 mg/ml and 31.25 mg/ml, respectively. Furthermore, it has been recently shown that antimicrobial activity of rice-based edible film incorporated with 3.2 % (w/v) ginger extract was preferable applied for anti-caries rice film with the bioactive compounds and essential oil that can exhibit the growth of bacteria. Rice film incorporated with 32 mg/mL ginger extract showed a significant antibacterial effect against *S. mutans* DMST 18777, which proved that ginger is being released from the film in the surrounding culture medium and that its antimicrobial activity has been preserved after the fortify in a polymer. The presence of phenolic compounds including 6- gingerol 6-shogaol, paradol, and zingerone, and essential oils including  $\alpha$ -curcumene,  $\alpha$ -zingiberene,  $\gamma$ -muurolene,  $\alpha$ -farnesene,  $\beta$ -bisabolene, and  $\beta$ -sesquiphellandrene in the rice-based edible film might be helpful for several therapeutic effects.

Thus, the development of rice-based edible film incorporated with dried ginger extract to the product may constitute an alternative way against the resistance to *S. mutans*.

## **Materials and Methods**

### **Raw material**

The fresh and 9 months matured rhizomes of ginger were obtained from the Hsu Chuan Foods Co., Ltd ([www.hcgroupthailand.com](http://www.hcgroupthailand.com)) in Chiang Rai, Thailand, which grown and controlled under Good Agricultural Practice ([www.hcgroupthailand.com](http://www.hcgroupthailand.com)). The identification was done according to Zingiberaceae expert and literature<sup>22</sup>. A voucher specimen (QBG No. 27329) was provided by the Queen Sirikit Botanic Garden (QBG), Chiang Mai, Thailand. The peeled gingers were washed in distilled water and cut into 0.2 x 4 x 0.4 cm. The cut gingers were then dried as the method reported earlier by Sida *et al.*<sup>8</sup>. Briefly, the cut gingers were placed in trays and put into the hot air dryer (Armfield, Hamshire, England) at 60°C for 308 min. The dried ginger was ground by hammer mill (Crompton, model 2000 Series, England) and sieved at 1.2 mm. The ginger powder was kept in the vacuum aluminum foil package and stored in a desiccator at ambient temperature for at least 24 h before further analysis.

### **Microwave-assisted extraction (MAE) experimental design**

The MAE was carried out on the laboratory microwave (Toshiba, Model ER-300C(S) Power Max 900, frequency  $2.45 \times 10^9$  Hz, Japan). A  $3^2$  full factorial design was constructed to investigate the influence of two variables, including microwave power at 400, 600, and 800 W and the reaction time at 1, 3, and 5 min (Table 1). The filtrate was collected and concentrated using a rotary evaporator under vacuum at  $50 \pm 4^\circ\text{C}$ , finally, dry extract yield was calculated and expressed in percentage. The dried extract samples were kept at  $4^\circ\text{C}$  until further used.

The experimental data were analyzed using the response surface regression procedure to fit the following second-order polynomial model (Eq. 1).

$$Y = \beta_0 + \sum_{i=1} \beta_i X_i + \sum_{i=1} \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j + e_0 \quad (1)$$

Where Y is the predicted response variable,  $\beta_0$  is the constant coefficient,  $\beta_i$  is the linear effect,  $\beta_{ii}$  is the squared effect,  $\beta_{ij}$  is interaction effects and  $X_i$  and  $X_j$  represent the independent variables respectively. Design-Expert version 6.0.10 (Stat-Ease Inc., Minneapolis, MN, USA) was applied to perform the experimental design and the data analysis.

### **Edible film preparation**

The rice-based film was prepared by casting technique according to Miksusanti *et al.*<sup>18</sup> with some modifications. For film formation, 7 g of glutinous Thai rice powder (Newgrade, Thaiwah Co., Ltd., Thailand) was dissolved in an aqueous solution (145 mL) of water, and heated at 1.6°C/min on a magnetic stirrer-hot plate. Sodium carboxymethyl cellulose 0.7 g and refined glycerine 1.75 mL were added slowly while continuous stirring on a hot plate. After entering the gelatinization stage, the ginger extract was added into Thai rice solution to reach a final concentration of 4, 8, 16, and 32 mg/mL, and then degas by sonication for 30 min. The rice-based film with ginger extract was then cast in a petri dish (9 cm diameter) and dried at 50°C for 6 h. The films were peeled off from the casing plates and conditioned for 7 days at 25°C in a desiccator before all analysis.

### **Total phenolic content (TPC)**

Total phenolic compounds were examined using the method described by Singleton & Rossi<sup>23</sup>. The ginger solution (200 µL) and 10% Folin-Ciocalteu reagent (1 mL) were mixed and 2% Na<sub>2</sub>CO<sub>3</sub> was then added with water: methanol (4:6) diluting solvent to make a total volume of 10 mL. Absorbance was recorded at 740 nm after 30 min using a spectrophotometer (UV-Vis model 1601, Shimadzu, Japan).

### **Antioxidant activities**

#### **DPPH radical-scavenging activity**

Four mL of extract solution and 1 mL of DPPH solution were mixed (0.1 mM in methanol) by a vortex mixer and then stood at room temperature in dark storage for 30 min<sup>24</sup>. The absorbance was recorded at 517 nm. The percentage of scavenging effect was calculated using the following equation (1) shown below:

$$\text{DPPH radical scavenging activity (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

where  $A_0$  was the absorbance of the control solution (DPPH without sample) and  $A_1$  was the absorbance of the ginger extract in DPPH solution.

### **ABTS method**

A mixture between 7 mM ABTS and 2.45 mM potassium persulphate, the ABTS solution, was stood in a dark place for 14±2 h before use. Afterward, the ABTS solution was diluted with ethanol to measure the absorbance of  $0.700 \pm 0.02$  at 734 nm<sup>25</sup>. Ginger extract (150 µL) was allowed to react with 4,850 µL of the ABTS solution for 6 min and then read by a spectrophotometer at 734 nm.

### **Ferric reducing ability power assay (FRAP assay)**

The FRAP assay was determined by the modification method from Benzie & Strain<sup>26</sup>. The FRAP solution (3 mL) was added to 150 µL of ginger extract for 10 min at 37 °C and the absorbance was recorded at 593 nm.

### **Antimicrobial activity**

#### **Cell culture condition**

*S. mutans* DMST 18777 (ATCC 251755) strain was obtained from Thailand Institute of Scientific and Technological Research. The strain was grown in Brain Heart Infusion Broth (BHI; Difco Laboratories, Sparks, MD, USA) at 37°C for 16-24 h under anaerobic condition.

#### **Evaluation of zone of inhibition**

The disc diffusion method was used to determine the zone of inhibition. The impregnated paper discs with 10 µL of ginger extracts and/or rice-based films (6 mm in diameter) were placed on

Mitis Salivarius agar plates, which were inoculated with *S. mutans* DMST 18777 according to the standard protocol described by the National Committee of Clinical Laboratory Standards (NCCLS)<sup>27</sup>. The plates were incubated at 37°C and the diameters of the inhibition zones were measured after 24 h. Filter paper discs containing DMSO without any test compounds served as a control and no inhibition was observed. Additionally, for comparative purposes, tetracycline (30 µg, 10 µL) was used as a reference standard. Each assay was performed in triplicate and repeated three times.

#### **Determination of minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)**

The MIC and MBC of the ginger extract against *S. mutans* DMST 18777 were determined by the reference protocol of the NCCLS method<sup>27</sup>. The concentrations of the ginger extracts used in these experiments ranged from 63 to 500 mg/mL. The densities of the cell suspensions of the respective microorganisms were adjusted to  $2.76 \times 10^6$  CFU/mL. The suspensions were transferred onto the plate and then incubated at 37°C for 24 h. The lowest concentration which inhibited the growth of the *S. mutans* DMST 18777 was taken as the MIC. While the MBC was defined as the lowest concentration that yielded no colony growth by sub-culturing on agar plates. All tests were carried out in triplicate.

#### **Analysis of active compounds in ginger using a high-performance liquid chromatography method (HPLC)**

After filtering through a 0.2 µm syringe filter, the final sample was used for injection. Standards of 6- gingerol, 6- shogaol, paradol, and zingerone were prepared. The method was performed on HPLC (HPLC, Agilent Technologies, Santa Clara, CA, USA) with a photodiode array detector. The HPLC system contained a C<sub>18</sub> reverse-phase column (Water C<sub>18</sub>, 250x4.6 mm, 5 µm particle size). The gradient elution was acetonitrile and water at a flow rate of 1.0 mL/min and detection of 282 nm. The mobile phase contained water (A) and acetonitrile (B). The

gradient elution program was set as follows: from 0 to 25 min, B was isocratic at 33%; from 25-35 min, B was changed from 33% to 55%; from 35 to 60 min, B followed changed linearly from 55% to 90%; from 60 to 65 min, B was a linear change from 90% to 33%; and from 65 to 70 min, B was isocratic at 33%<sup>28</sup>.

### **Headspace solid-phase microextraction (HS-SPME)**

To evaluate the volatile compounds of the rice edible film incorporated with crude ginger extract, the compounds were extracted using carboxen/polydimethylsiloxane (CAR/PDMS) fiber. The sample headspace (5 g) was transferred into a 25 mL screw cap glass vial and extracted at 50 °C for 30 min. The bound volatiles was injected into GC-MS analysis<sup>29</sup>.

The measurement of the target analysts was performed using GC-MS (GC-17A, Shimadzu, Japan) coupled with mass spectrometry (QP 5050A, Shimadzu, Japan). Capillary BPX-5 (30 m × 0.25 mm × 1.00 μm; SGE, Melbourne, Australia) column was used for separation and run at 1.0 mL/min with helium as the carrier gas. The inlet temperature was 250 °C in split mode (1:50). The initially oven temperature was started from 80 °C for 1 min, heated to 220 °C at 5 °C/min and maintained for 10 min, and finally increased to 250 °C. The detector temperature was set at 300 °C.

### **Statistical analysis**

All experiments were carried out according to the relevant guidelines and regulation. The data were shown as the mean and standard deviation for the triplicate analyses. The mean comparisons of the physical and chemical properties (yield, TPC, DPPH, ABTS, FRAP, and bioactive compounds) were analyzed using ANOVA in SPSS version 17.0 (SPSS Inc., Chicago, USA). Statistical significance was analyzed at  $p \leq 0.05$  using Duncan's multiple range tests.

### **Data availability**

The data generated during the current study are available from the corresponding author on reasonable request.

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### **Author contributions**

A. Kawee-ai interpreted the results and write the manuscript, S. Sida conducted and collected test data and interpreted the results, P. Wanachantararak analyses antimicrobial activity, N. Utama-ang designed and managed the experiments, reviewed and revised the manuscript.

### **Conflicts of interest**

All authors declared no conflicts of interest.

### **Figure Legends**

**Figure 1.** Contour plots of the interaction between power and time on (A) yield, (B) TPC, (C) ABTS, (D) FRAP and (E) 6-gingerol.

**Figure 2.** Overlay plot for the optimal condition of microwave-assisted ginger extraction.

**Figure 3.** Diagram of the 6-gingerol conversion into 6-shogaol, paradol and zingerone through the heating and radiation of the power of the microwave process.

**Table 1** Operation parameters and the responses for microwave-assisted ginger extraction by 3<sup>2</sup> full factorial design.

Microwave		Yield (%, dw) <sup>*,**</sup>	TPC (mg GAE/g)	Antioxidant activity			Bioactive compounds			
Power (W)	Time (min)			DPPH (%inhibition)	ABTS (mgTrolox/g)	FRAP (mgTrolox/g)	6-Gingerol (mg/g)	6-Shogaol (mg/g)	Paradol (mg/g)	Zingerone (mg/g)
400	1	7.57±0.50 <sup>b</sup>	200.84±4.54 <sup>a</sup>	91.87±0.42 <sup>a</sup>	104.21±3.08 <sup>b</sup>	297.36±13.16 <sup>b</sup>	73.41±1.33 <sup>a</sup>	12.84±0.78 <sup>bc</sup>	23.51±0.87 <sup>a</sup>	4.99±0.26 <sup>bc</sup>
600	1	9.32±1.29 <sup>a</sup>	119.93±5.66 <sup>d</sup>	89.21±0.98 <sup>b</sup>	93.37±4.58 <sup>c</sup>	296.64±2.74 <sup>b</sup>	60.39±2.46 <sup>bc</sup>	12.50±1.84 <sup>bc</sup>	25.54±7.86 <sup>a</sup>	5.15±0.16 <sup>b</sup>
800	1	10.21±0.45 <sup>a</sup>	111.32±5.49 <sup>e</sup>	81.53±2.45 <sup>c</sup>	81.02±5.95 <sup>de</sup>	267.95±1.41 <sup>d</sup>	56.11±1.22 <sup>bcd</sup>	10.90±0.63 <sup>bc</sup>	17.91±0.59 <sup>ab</sup>	4.67±0.24 <sup>cd</sup>
400	3	9.88±0.54 <sup>a</sup>	132.20±5.43 <sup>c</sup>	84.32±0.22 <sup>d</sup>	83.42±7.35 <sup>d</sup>	278.43±2.31 <sup>c</sup>	56.48±1.39 <sup>bcd</sup>	11.89±0.26 <sup>bc</sup>	19.98±2.96 <sup>ab</sup>	4.57±0.04 <sup>cde</sup>
600	3	10.47±0.10 <sup>a</sup>	104.95±0.95 <sup>e</sup>	89.28±0.94 <sup>c</sup>	72.69±3.72 <sup>f</sup>	245.91±2.57 <sup>e</sup>	47.23±1.31 <sup>d</sup>	9.94±1.89 <sup>c</sup>	23.69±1.42 <sup>b</sup>	4.35±0.14 <sup>de</sup>
800	3	9.73±0.19 <sup>a</sup>	109.84±1.01 <sup>e</sup>	85.80±0.51 <sup>cd</sup>	86.67±2.27 <sup>cd</sup>	282.25±1.79 <sup>c</sup>	54.38±1.88 <sup>bcd</sup>	11.80±2.02 <sup>bc</sup>	16.65±1.09 <sup>b</sup>	4.34±0.18 <sup>de</sup>
400	5	10.42±0.70 <sup>a</sup>	111.13±1.34 <sup>e</sup>	85.84±0.27 <sup>cd</sup>	87.29±5.09 <sup>cd</sup>	284.07±2.67 <sup>c</sup>	57.40±2.18 <sup>bcd</sup>	13.18±0.75 <sup>b</sup>	20.40±1.21 <sup>ab</sup>	4.99±0.30 <sup>bc</sup>
600	5	10.70±1.31 <sup>a</sup>	119.87±5.49 <sup>d</sup>	86.84±1.25 <sup>d</sup>	74.68±1.89 <sup>ef</sup>	266.90±2.22 <sup>d</sup>	51.12±1.69 <sup>cd</sup>	10.29±0.79 <sup>bc</sup>	16.60±2.05 <sup>b</sup>	4.12±0.52 <sup>e</sup>
800	5	7.31±1.01 <sup>b</sup>	145.31±1.82 <sup>b</sup>	89.52±0.73 <sup>b</sup>	120.86±2.33 <sup>a</sup>	377.86±4.29 <sup>a</sup>	64.76±2.52 <sup>ab</sup>	16.04±1.86 <sup>a</sup>	19.31±1.29 <sup>ab</sup>	5.79±0.06 <sup>a</sup>

\* dw means dry weight

\*\* a-f represented the significant difference in the columns at  $p < 0.05$

**Table 2** Regression coefficient of the models of extraction yield, TPC, ABTS, FRAP and 6-Gingerol.

Response	Final equation in terms of actual factors	$R^2$	$P$ -value
Extraction yield (%)	+ 2.66 + 0.01 * Power <sup>ns</sup> + 2.33 * Time <sup>ns</sup> - 3.66×10 <sup>-3</sup> * Power * Time	0.7044	0.0498
TPC (mg GAE/g)	+ 512.18 - 0.90 * Power - 76.19 * Time + 5.07×10 <sup>-4</sup> * Power <sup>2</sup> + 4.34 * Time <sup>2</sup> + 0.07 * Power * Time	0.9758	0.0025
ABTS (mgTrolox/g)	+ 205.36 - 0.25 * Power <sup>ns</sup> - 35.47 * Time <sup>ns</sup> + 1.43×10 <sup>-4</sup> * Power <sup>2, ns</sup> + 2.77 * Time <sup>2</sup> + 0.03 * Power * Time	0.9267	0.0218
FRAP (mgTrolox/g)	+ 710.63 - 1.21 * Power <sup>ns</sup> - 82.80 * Time <sup>ns</sup> + 8.50×10 <sup>-4</sup> * Power <sup>2</sup> + 6.77 * Time <sup>2, ns</sup> + 0.08 * Power * Time	0.9005	0.0391
6-Gingerol (mg/g)	+ 148.88 - 0.22 * Power <sup>ns</sup> - 20.12 * Time <sup>ns</sup> + 1.43×10 <sup>-4</sup> * Power <sup>2</sup> + 1.76 * Time <sup>2</sup> + 0.01 * Power * Time	0.9267	0.0218

\* ns means not significant at  $p < 0.05$

**Table 3** Predicted and actual values of optimal conditions

Response	Actual value	Predicted value	% Error
Yield (%)	7.66±0.66	7.61	0.65
TPC (mg GAE/g)	198.24±0.74	193.93	2.17
DPPH (%)	91.39±0.69	89.00	2.61
ABTS (mgTrolox/g)	106.42±3.13	106.00	0.39
FRAP (mgTrolox/g)	304.62±5.49	317.98	-4.38
6-Gingerol (mg/g)	71.57±3.60	71.52	0.07
6-Shogaol (mg/g)	12.52±1.03	12.78	-2.07
Paradol (mg/g)	23.11±1.16	24.76	-7.13
Zingerone (mg/g)	5.07±0.32	5.24	-3.35

**Table 4** Antimicrobial activity of crude ginger extracts and rice-based edible film at different concentrations against *S. mutans* by disc diffusion method.

Sample	Concentration (mg/mL)	Inhibition zone (mm)
DMSO (Negative control)		-
Crude ginger extract <sup>ns*</sup>	63	11.00±1.41
	125	11.00±0.01
	250	10.50±0.71
	500	9.50±0.71
MIC** (mg/mL)	0.49	
MBC** (mg/mL)	31.25	
DMSO (Negative control)		-
Tetracycline 30 µg (Positive control)		41.20±0.47 <sup>a</sup>
Rice film+ginger extract	0	-
	4.0	-
	8.0	-
	16.0	-
	32.0	12.69±0.07 <sup>b</sup>

\* ns means not significantly different at  $p \leq 0.05$

\*\* MIC is Minimum inhibitory concentration; MBC is Minimum bactericidal concentration.

**Table 5** The phenolic compounds, antioxidant activity and volatile compounds of rice strip film incorporated with various crude ginger extract

Investigate parameters	Unit	Ginger extract concentration in rice film (% w/v)				
		0	0.4	0.8	1.6	3.2
TPC	mg GAE/100 g	-	415.84±10.95 <sup>d,**</sup>	559.45±5.58 <sup>c</sup>	992.69±7.30 <sup>b</sup>	1,613.35±7.30 <sup>a</sup>
Antioxidant activity		-				
DPPH	mgTrolox/ 100 g	-	392.08±47.83 <sup>d</sup>	939.11±65.96 <sup>c</sup>	1,732.99±51.00 <sup>b</sup>	2,637.82±88.38 <sup>a</sup>
ABTS	mgTrolox/ 100 g	-	347.07±41.43 <sup>d</sup>	671.72±56.16 <sup>c</sup>	865.04±21.89 <sup>b</sup>	1,634.70±72.86 <sup>a</sup>
FRAP	mgTrolox/ 100 g	-	1,060.19±50.45 <sup>d</sup>	2,137.86±66.74 <sup>c</sup>	3,346.59±75.67 <sup>b</sup>	6,244.64±90.95 <sup>a</sup>
Bioactive compounds						
6-Gingerol	mg/g	-	.42±0.02 <sup>c</sup>	1.96±0.07 <sup>c</sup>	7.11±0.10 <sup>b</sup>	12.90±0.39 <sup>a</sup>
6-Shogaol	mg/g	-	0.51±0.01 <sup>c</sup>	0.65±0.01 <sup>c</sup>	2.13±0.13 <sup>b</sup>	5.52±0.95 <sup>a</sup>
Paradol	mg/g	-	ND	0.85±0.01 <sup>c</sup>	1.43±0.04 <sup>b</sup>	1.86±0.11 <sup>a</sup>
Zingerone	mg/g	-	ND	0.23±0.01 <sup>c</sup>	0.43±0.01 <sup>b</sup>	0.69±0.03 <sup>a</sup>
Volatile compounds						
$\alpha$ -Curcumene <sup>ns*</sup>	%	-	16.86±8.93	13.29±2.09	14.51±2.91	13.85±1.54
$\alpha$ -Zingiberene <sup>ns</sup>	%	-	27.06±4.29	30.81±4.84	33.23±5.85	34.60±6.69
$\gamma$ -Muurolene <sup>ns</sup>	%	-	7.64±1.21	6.30±0.37	6.83±1.03	6.56±1.03
$\alpha$ -Farnesene <sup>ns</sup>	%	-	10.36±1.63	10.52±1.02	12.64±2.61	12.77±2.01
$\beta$ -Bisabolene <sup>ns</sup>	%	-	8.69±1.06	6.74±1.06	6.60±1.66	6.27±2.24
$\beta$ -Sesquiphellandrene <sup>ns</sup>	%	-	15.38±2.41	14.11±2.22	13.30±3.97	13.13±4.58

\* ns means not significant at  $p < 0.05$

\*\* a-d represented the significant difference in the rows at  $p < 0.05$

# Figures

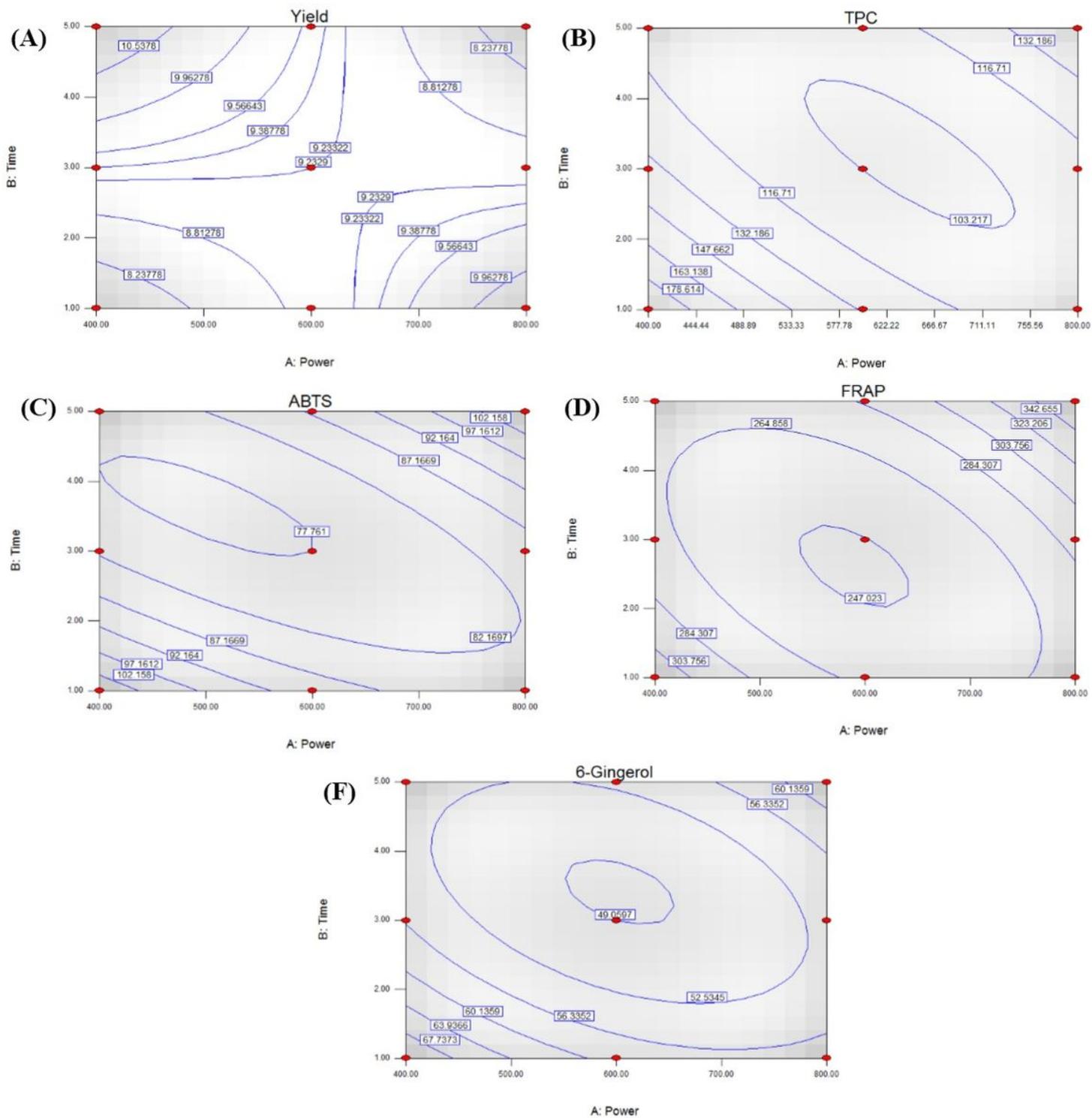
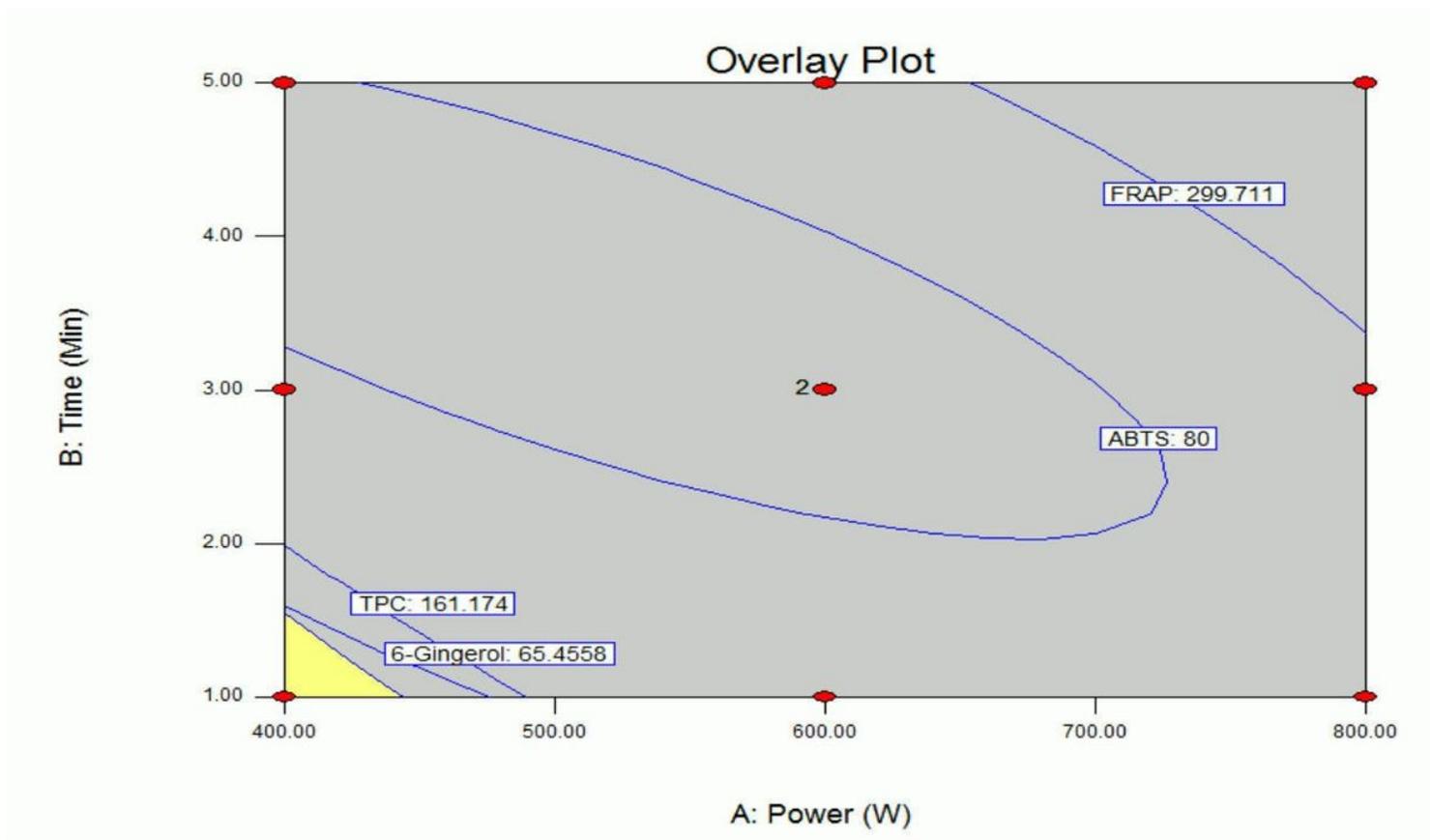


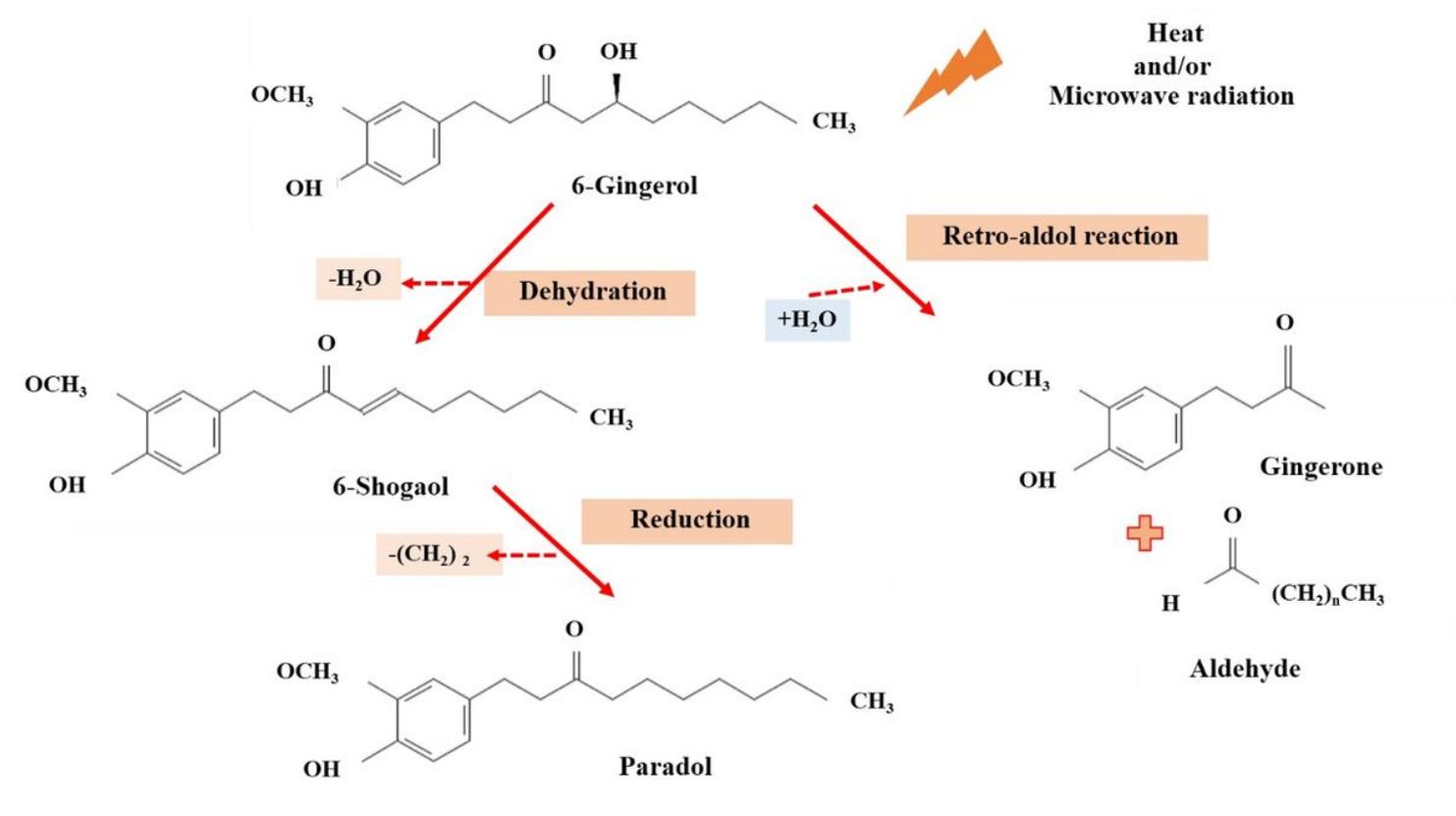
Figure 1

Contour plots of the interaction between power and time on (A) yield, (B) TPC, (C) ABTS, (D) FRAP and (E) 6-gingerol.



**Figure 2**

Overlay plot for the optimal condition of microwave-assisted ginger extraction.



### Figure 3

Diagram of the 6-gingerol conversion into 6-shogaol, paradol and zingerone through the heating and radiation of the power of the microwave process.