Determination of Gingerols and Shogaols Content from Ginger (Zingiber officinale Rosc.) through Microwave-Assisted Extraction

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Abstract: Ginger (Zingiber officinale Rosc.) is a plant recognized for its pungent taste and aromatic qualities, primarily derived from its underground rhizome. Apart from its widespread culinary applications, ginger is valued for its potential health benefits attributed to the presence of gingerols and shogaols. For this reason, this work proposes the development of a microwave-assisted extraction (MAE) method for the extraction of gingerols and shogaols present in ginger rhizomes. The influence of the extraction temperature (50–100 °C), the solvent composition (50–100% ethanol in water), and the sample-to-solvent ratio (0.3–0.7 g sample: 20 mL) on the extraction of these bioactive compounds has been studied. To this end, a Box–Behnken experimental design (BBD) in combination with a response surface methodology (RSM) has been applied. The optimum conditions for the total extraction of gingerols and shogaols were: 87% ethanol in water, 100 °C, and 0.431 g of ginger sample in 20 mL solvent. The developed method required short extraction times (5 min) and demonstrated favorable levels of repeatability and intermediate precision (CV < 5%). Finally, the MAE method was successfully used for the extraction of gingerols and shogaols from a variety of ginger samples.

Keywords: Box–Behnken design; gingerols; microwave-assisted extraction; response surface methodology; shogaol; UHPLC-QToF-MS; Zingiber officinale Rosc.

1. Introduction

Ginger, scientifically known as Zingiber officinale Rosc., is a plant belonging to the Zingiberaceae family whose underground rhizome is highly valued for its spicy flavor and aroma [1,2]. It is believed to be an original from Southern China or India, where it has been used in traditional medicine for decades [2]. Therefore, in addition to its culinary use, ginger is also highly valued for its medicinal properties. More precisely, the beneficial effects of ginger are largely attributed to the presence of gingerols and their analogues, shogaols, which belong to the family of molecules that contain a vanillyl group, known as “vanilloids”. These compounds present certain chemical similarities with capsaicin, piperine, and vanillin, as they all have a “vanillyl” functional group [3–6]. Gingerols are the most abundant pungent compounds in fresh ginger roots, where they are found with different chain lengths, mainly 6-gingerol, 8-gingerol, or 10-gingerol [7,8]. Several research studies have demonstrated that the capacity of gingerols to control pain through the VR1 receptor is linked to the size of the side chain [9,10]. Gingerols are prone to
dehydration as they contain a β-hydroxy keto group, which turns into the corresponding shogaols: 6-shogaol, 8-shogaol and 10-shogaol [11]. Different investigations have shown that these pungent compounds present in ginger have anticancer, analgesic, and anti-inflammatory properties [12,13].

For this reason, it is important to use an appropriate extraction method that enables the extraction of these molecules of interest [14]. Among the existing extraction techniques, MAE stands out as a method that allows performing extractions in short times with good yields and low solvent consumption [15]. MAE is based on the transformation of electromagnetic energy into thermal energy, i.e., heat transfer. More specifically, electromagnetic waves are formed by an electric and a magnetic field that oscillate perpendicularly to each other. When there are polar molecules or ions in the extraction solvent, these molecules move to get oriented according to the oscillating field [14,16]. These movements, which are known as dipolar rotation and ionic friction, cause rapid heating [17]. Therefore, the dielectric properties of the solvent used are an important factor to take into account for extraction purposes. Certain solvents, such as methanol, ethanol, or water, are extensively used as they present suitable dielectric properties that allow them to transform electromagnetic energy into heat and subsequently diffuse the generated heat into the surrounding molecules [18]. MAE has previously been studied for the extraction of 6-gingerol and 6-shogaol [19,20], but has not been evaluated for the extraction of the other major gingerols and shogaols from ginger (8-gingerol, 10-gingerol, 8-shogaol, and 10-shogaol).

Although MAE offers all the above-mentioned advantages, it is important to understand how certain extraction factors, such as the conditions of the solvent used, may affect the extraction of the bioactive compounds. RSM, combined with the design of experiments (DOE), has been chosen to explore these factors. RSM consists of a set of statistical techniques that aim to determine which extraction factors or combinations of factors may affect the response variable as well as identify the optimal conditions of the extraction process [21]. Concerning DOE, a BBD was used for the present study. BBD demonstrates several advantages compared to other DOE methodologies. These include the ability to assess the interaction effects between factors, reduced experimental requirements (resulting in cost savings), and the exclusion of extreme points on the cubic region plot. Instead, the points are strategically positioned around the mid-values of the edges and the center of the cube [22].

For all these reasons, this work intends to develop a MAE method for the major bioactive and pungent compounds obtained from ginger: gingerols and shogaols. By combining BBD with RSM, the effect of the following extraction factors on the extraction of these compounds will be determined: extraction temperature, characteristics of the extraction solvent used (percentage of ethanol and water), and ratio between the ginger sample and the volume of solvent used. Finally, the applicability of the developed and optimized MAE method was assessed on a variety of ginger samples of different origins and chemical compositions.

2. Materials and Methods
2.1. Natural Matrix and Reagents Used

Given the previously mentioned importance of ginger, this study will use ginger purchased at a local market in Veracruz (Veracruz, Mexico), precisely situated at coordinates 19°12′41.1″ N; 96°09′41.3″ W. To increase the contact surface between the ginger sample and the extraction solvent and thus favor the extraction of the compounds of interest, the ginger sample was subjected to pre-treatment. Such pre-treatment, which allows transforming fresh ginger into ginger powder, has been previously described in detail in other previous studies by our research group [23]. In addition to the ginger samples from Mexico, the optimized extraction method was tested on other ginger samples collected from Spain, Germany, and Morocco.

Some of the reagents employed in the study consisted of acetonitrile, methanol, and ethanol, all 99.9% pure, HPLC-grade, and acquired from Panreac Química S.L.U. (Castel-
lar del Vallés, Barcelona, Spain). For acidification of the mobile phases in the UHPLC, glacial acetic acid (99% HPLC-grade) purchased from Merck (Darmstadt, Germany) was used. Milli-Q water, acquired from a Millipore water purification system (Bedford, MA, USA), was employed for both extraction and chromatographic analysis purposes. The quantification of the bioactive compounds of interest was conducted using 6-gingerol (5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone) and 6-shogaol (1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one) as commercial standards, obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Microwave-Assisted Extraction

A MARS 6 One Touch Technology system (1800 W) (CEM Corporation, Matthews, NC, USA) was employed to perform the microwave-assisted extraction.

The extraction procedure entailed introducing the corresponding quantity of ginger powder into a 75 mL MARSXpress tube (CEM Corporation), followed by the addition of 20 mL of an ethanol:water solution with varying concentrations. The tubes were sealed using their safety caps and placed inside the microwave oven. To perform the BBD, different extraction conditions were tested by varying the following factors: extraction solvent composition (50–100% ethanol in water), extraction temperature (50–100 °C), and sample-to-solvent ratio (0.3–0.7 g sample: 20 mL). The extraction time used remained constant at 10 min, including a 5 min heating ramp at 800 W and an additional cooling period of 5 min. Following the completion of the extractions, the obtained extracts underwent centrifugation at a speed of 1790 × g for a duration of 5 min. The resulting supernatant was made up to a constant final volume of 25 mL for all extracts. Lastly, the extracts obtained were filtered using nylon syringe filters (0.22 µm) before their identification by ultra-high-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (UHPLC-Q-ToF-MS) and their quantification by ultra-high-performance liquid chromatography coupled to a diode array detector (UHPLC-DAD).

The optimal range of ethanol percentages in water has been studied. The percentages tested were 0; 20; 40; 60; 80; and 100% ethanol–water. Other parameters were extraction time: 10 min; sample-to-solvent ratio: 0.5 g:20 mL; and temperature: 75 °C. In this sense, the extraction temperature range has also been evaluated. The temperatures tested were 50, 75, 100, 125, and 150 °C. Other parameters were extraction time: 10 min; sample-to-solvent ratio: 0.5 g:20 mL; and ethanol percentage: 75%.

2.3. Gingerols and Shogaols Analyzed and Quantified via UHPLC-Q-ToF-MS

The identification of gingerols and shogaols within the ginger extracts was accomplished using UHPLC-Q-ToF-MS analysis. The analytical method previously developed by our research group [23] was used. The individual identification of the compounds relied on their distinctive elution order and their molecular weight [M+Na]⁺, as they form adducts with Na. A comprehensive list of the identified compounds can be found in Table 1.

Table 1. Retention time (min), m/z [M+Na]⁺, calibration curve, and R² of the calibration curve of each of the major gingerols and shogaols identified in ginger by means of UHPLC-QToF-MS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>m/z</th>
<th>Retention Time (min)</th>
<th>Calibration Curve</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-gingerol</td>
<td>6-G</td>
<td>294.4</td>
<td>1.88</td>
<td>y = 3335.6x – 2574.5</td>
<td>0.9986</td>
</tr>
<tr>
<td>6-shogaol</td>
<td>6-S</td>
<td>276.4</td>
<td>3.69</td>
<td>y = 1839x – 2241.5</td>
<td>0.9973</td>
</tr>
<tr>
<td>8-gingerol</td>
<td>8-G</td>
<td>322.4</td>
<td>3.79</td>
<td>y = 3335.6x – 2574.5</td>
<td>0.9986</td>
</tr>
<tr>
<td>8-shogaol</td>
<td>8-S</td>
<td>304.4</td>
<td>4.78</td>
<td>y = 1839x – 2241.5</td>
<td>0.9973</td>
</tr>
<tr>
<td>10-gingerol</td>
<td>10-G</td>
<td>350.5</td>
<td>4.80</td>
<td>y = 3335.6x – 2574.5</td>
<td>0.9986</td>
</tr>
<tr>
<td>10-shogaol</td>
<td>10-S</td>
<td>332.5</td>
<td>5.52</td>
<td>y = 1839x – 2241.5</td>
<td>0.9973</td>
</tr>
</tbody>
</table>

Once the gingerols and shogaols in the ginger extracts had been identified, Ultra-High-Performance Liquid Chromatography (UHPLC) equipment (Waters Corp., ACQUITY™, UHPLC™ H-Class; Milford, MA, USA) was used for their analysis and quantification. The
UHPLC system utilized in the study was comprised of a quaternary pump, an automatic sampler, and a column oven maintained at a temperature of 65 °C. Additionally, it was coupled with a DAD from Waters Corp. (PDA100; Milford, MA, USA). The chromatographic conditions used were based on the method described by Vázquez-Espinosa et al., with slight adjustments [24].

The 6-G and 6-S compounds were quantified by plotting a calibration line for each of the standards, as shown in Table 1. For this purpose, each of the standards was injected at different specific concentrations within the range of 0.1–100 mg L\(^{-1}\). Due to the analogous chemical structures of the other compounds and their corresponding similar absorbance characteristics, quantification was performed utilizing these calibration curves. The proportionate molecular mass of each compound was considered during quantification. Specifically, for 8-G and 10-G gingerols, quantification was based on the calibration curve of 6-G, whereas for 8-S and 10-S shogaols, quantification relied on the calibration curve of 6-S.

2.4. Experimental Design: BBD

For the present study, BBD was the design of the experiment used to determine the optimal extraction conditions. BBD is characterized by the fact that each of the extraction factors is studied at three different levels: a lower level (−1), an intermediate level (0), and a higher level (+1). The extraction factors that were specifically studied included: percentage of ethanol in water (solvent) \((X_1)\), extraction temperature \((X_2)\), and sample-to-solvent ratio \((X_3)\). The response variable to be optimized was the total gingerol and shogaol content \(Y_{TGSC}\), calculated as the sum of the concentrations of each of the 6 compounds identified in ginger (6-G, 6-S, 8-G, 8-S, 10-G, and 10-S). The Box–Behnken design comprised a total of 15 experiments performed in duplicate with three replicates at the center point \((0, 0, 0)\).

The RSM was used to obtain a regression model based on the results from the BBD. This model included a second-order polynomial equation (Equation (1)), which allowed for the establishment of correlations between the independent variables and the response variable. To evaluate the suitability of the model developed, an analysis of variance (ANOVA) was conducted.

\[
y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + r, \tag{1}
\]

where \(y\) is the predicted response \(Y_{TGSC}\); \(\beta_0\) is the model constant; \(X_i\) and \(X_j\) are the independent variables; \(\beta_i\) are the linear coefficients; \(\beta_{ii}\) are the coefficients corresponding to the interactions; \(\beta_{ij}\) are the quadratic coefficients; and \(r\) is the sum of the mean squares of the error. The statistical analyses were conducted following those described by Gonzalez-Gonzalez et al. [23] through the software application Statgraphic Centurion Version XVIII (Statgraphics Technologies, Inc., Los Llanos, VA, USA).

3. Results and Discussion

3.1. Establishing the Optimal Range of Ethanol Percentage in Water to Be Used as a Solvent

The extraction solvent polarity, influenced by the ethanol percentage in water, can impact the transfer of gingerols and shogaols from the solid matrix to the liquid phase, consequently influencing the yields obtained through MAE. Therefore, to select the percentage range to be used for the BBD, a univariate study was conducted using different ethanol-to-water ratios. The percentages tested were 0; 20; 40; 60; 80; and 100% ethanol–water for an extraction time of 10 min, while the rest of the extraction factors remained at intermediate values of the MAE experimental design \(0.5 \text{ g:20 mL; } 75 \degree \text{ C}\). The extractions were performed in triplicate, and the results obtained are shown in Figure 1. From the results obtained, it can be observed that as the percentage of ethanol in water increased, the total amount of gingerols and shogaols extracted also increased. This may indicate that as the percentage of ethanol in water increases, the polarity of the extraction solvent becomes more similar to that of the compounds of interest, which favors their extraction [23]. This
result is in agreement with other authors who also used high percentages of ethanol to extract gingerols and shogaols from ginger using MAE [19,25]. Based on these results, a range of 50–100% EtOH in water was selected for the design.

![Figure 1. Amount of total gingerol and shogaol compounds (mg Y\text{\textsubscript{TGSC}} g\textsuperscript{-1} sample) extracted by MAE at different mixtures of ethanol in water (0, 25, 50, 75, and 100%) (n = 3). Statistical analysis using Tukey’s test at the 95% significance level revealed significant differences between the groups, indicated by different letters (a–d).](image)

3.2. Establishing the Extraction Temperature Range

The extraction temperature is a factor that may affect either positively or negatively the final yields of bioactive compounds by facilitating their recovery or impeding it through the degradation of the thermolabile compounds of interest [26]. Therefore, to select the range of temperatures to be used in the BBD, a univariate study was conducted at different temperatures. The temperatures studied were 50, 75, 100, 125, and 150 °C for an extraction time of 10 min, while the rest of the extraction variables were maintained at intermediate conditions (0.5 g:20 mL; 75% ethanol in water). This temperature range was selected based on our research group’s knowledge of compounds similar to gingerols, such as capsaicinoids [27], as well as stability studies of gingerols at high temperatures [28,29]. The extraction process was conducted in triplicate, and the outcomes derived from these extractions are presented in Figure 2. From Figure 2, there were no significant differences between the Y\text{\textsubscript{TGSC}} extracted within the range of temperatures evaluated. However, at temperatures above 100 °C, a variation in the color of the extract obtained could be observed, with a brownish coloration attributable to the degradation of certain other compounds in the sample as a consequence of the temperature. Based on the experimental findings, no significant differences were observed in the Y\text{\textsubscript{TGSC}} extracted at various temperatures. Furthermore, considering the degradation of compounds at temperatures above 100 °C, the substantial energy demands associated with high temperatures, and the prolonged cooling waiting times after extraction, it has been established that the operational range should be between 50 and 100 °C.

3.3. Optimizing the Conditions for Microwave-Assisted Extractions

Once the univariate study was completed for the temperature and the percentage of ethanol in water, the operational range for the sample-to-solvent ratio in the BBD was determined according to the team’s experience [30–32]. Table 2 shows the ranges used for each of the factors studied.
A polynomial equation (Equation (2)) was constructed based on the results obtained together with the predicted values obtained through the polynomic equation. The results of these analyses (Table 4) revealed both the individual and combined effects of the extraction factors on the response variable (YTGSC). Specifically, the factors with p-values lower than 0.05 (95% significance level) had a relevant influence on the response. This information was visually complemented using a Pareto chart (Figure 3). In this chart, it can be seen that the factors whose horizontal bar exceeded the vertical line were those considered significant concerning the response variable.

Based on the findings obtained from both the Pareto chart and the ANOVA analysis, it was determined that the only factor that exhibited a substantial impact on the extraction process was the quadratic effect of the ethanol percentage in water. This result is consistent with other works found in the bibliography, where the extraction of gingerols and shogaols [33,34], as well as analogous compounds with vanilloid rings like those of capsaicinoids [26], have been assessed. In this work, it is determined that the extraction solvent is one of the most influential parameters for extracting this type of compound. To extract natural compounds, solvents or mixtures of solvents that have a similar polarity to the compounds to be extracted must be used. It can, therefore, be concluded that the concentration of the extraction solvent is the most influential parameter concerning the extraction of gingerols and shogaols. Hence, solvents or mixtures of solvents that can solubilize the compounds of interest should be used. In the case of gingerols and shogaols, high percentages of methanol or ethanol in water are usually used, as obtained in this work [20,25]. Finally, a polynomial equation (Equation (2)) was constructed based on the

![Figure 2. Amount of total gingerol and shogaol compounds (mg YTGSC g⁻¹ sample) extracted by MAE at different temperatures (50, 75, 100, 125, and 150 °C) (n = 3). Statistical analysis using Tukey’s test at a significance level of 95% revealed no significant differences between the groups (same letters).](image-url)
coefficients of each of the factors and their interactions to correlate the response variable with each of the factors and calculate the predicted values (Table 4).

\[
Y_{\text{TGSC}} = 19.5753 + 1.69294 \cdot X_1 + 0.713704 \cdot X_2 - 0.436851 \cdot X_3 - 3.72328 \cdot X_1^2 + 1.47233 \cdot X_1 \cdot X_2 - 1.28753 \cdot X_1 \cdot X_3 + 1.29497 \cdot X_2^2 + 0.14215 \cdot X_2 \cdot X_3 - 1.34295 \cdot X_3^2.
\] (2)

Table 3. Box–Behnken experimental design for total gingerol and shogaol compounds (mg Y$_{\text{TGSC}}$ g$^{-1}$ sample) extracted by MAE. Experimental and predicted results are included.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Factors</th>
<th>Responses Y$_{\text{TGSC}}$ (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. ANOVA results obtained (quadratic model) for total gingerol and shogaol compounds extracted by MAE using a BBD.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Coefficients</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$: %EtOH</td>
<td>22.928</td>
<td>1</td>
<td>19.5753</td>
<td>22.928</td>
<td>6.15</td>
<td>0.055</td>
</tr>
<tr>
<td>$X_2$: Temperature</td>
<td>4.074</td>
<td>1</td>
<td>1.69294</td>
<td>4.074</td>
<td>1.09</td>
<td>0.343</td>
</tr>
<tr>
<td>$X_3$: Ratio</td>
<td>1.526</td>
<td>1</td>
<td>0.713704</td>
<td>1.526</td>
<td>0.41</td>
<td>0.550</td>
</tr>
<tr>
<td>$X_1X_1$</td>
<td>51.185</td>
<td>1</td>
<td>-0.436851</td>
<td>51.185</td>
<td>13.73</td>
<td>0.013</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>8.671</td>
<td>1</td>
<td>-3.72328</td>
<td>8.671</td>
<td>2.33</td>
<td>0.187</td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>6.630</td>
<td>1</td>
<td>1.47233</td>
<td>6.630</td>
<td>1.78</td>
<td>0.239</td>
</tr>
<tr>
<td>$X_2X_2$</td>
<td>6.191</td>
<td>1</td>
<td>-1.28753</td>
<td>6.191</td>
<td>1.66</td>
<td>0.253</td>
</tr>
<tr>
<td>$X_2X_3$</td>
<td>0.080</td>
<td>1</td>
<td>1.29497</td>
<td>0.080</td>
<td>0.02</td>
<td>0.888</td>
</tr>
<tr>
<td>$X_3X_3$</td>
<td>6.65916</td>
<td>1</td>
<td>0.14215</td>
<td>6.65916</td>
<td>1.79</td>
<td>0.239</td>
</tr>
<tr>
<td>Error total</td>
<td>18.6437</td>
<td>5</td>
<td>-1.34295</td>
<td>3.72873</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (corr.)</td>
<td>128.137</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4. Optimal Extraction Conditions

In addition to the aforementioned information, the response surface methodology (RSM) facilitated the determination of the optimal values for the independent factors, which would lead to the attainment of the most favorable results for the response variable. Table 5 presents these optimum values that would yield the highest Y$_{\text{TGSC}}$ extractions from the ethanolic extract obtained from the ginger rhizome.
null
technique has a shorter extraction time, which reduces energy consumption and solvent requirements. High extraction efficiency was achieved because thermal stresses and high localized pressure created surface creases and ruptures that allowed the solvent to enter easily into cellular channels [41]. In recent years, MAE water extraction combined with steam explosion has been used for the extraction of valuable compounds from black alder bark with very good results [42]. This technique would be interesting to apply to ginger to obtain extracts free of organic solvents.

3.5. Optimization of Extraction Time for Maximum Recoveries

Upon establishing the optimal extraction conditions, it became imperative to identify the ideal extraction time that would yield the highest concentration of total gingerols and shogaols from ginger rhizome within the shortest timeframe possible. To accomplish this, triplicate extractions were performed, varying the extraction time from 5 to 30 min. The findings from these extractions are depicted in Figure 4. Interestingly, no significant disparities were observed among the different extraction durations examined. As a result, an optimal extraction time of 5 min was chosen, as it yielded comparable outcomes to longer extraction times. This choice offers notable advantages in terms of cost and labor efficiency.

![Figure 4. Total gingerol and shogaol compounds (mg YTGSC g⁻¹ sample), extracted by the optimal conditions obtained by MAE at different extraction times (5, 10, 15, 20, 25, and 30 min) (n = 3). Statistical analysis using Tukey’s test at a significance level of 95% revealed no significant differences between the groups (same letters).](image)

3.6. Precision Evaluation of the Optimized MAE Method

Ultimately, the precision of the method was evaluated through the examination of both repeatability and intermediate precision. To assess the precision of the method, a series of 27 extractions were conducted over the course of three consecutive days. The extractions were performed utilizing the established optimal values (Table 5) obtained during the development of the MAE method. Repeatability was evaluated by calculating the coefficient of variation (CV) based on experiments conducted on the same day, while intermediate precision was determined by examining the CV of experiments carried out on different days. A repeatability CV of 4.85% and a CV of 5.71% for intermediate precision were obtained. These data are within the acceptable limits defined by the Association of Analytical Communities (AOAC International) [43], so the developed MAE method was confirmed to present good precision.
3.7. Applying the Optimum MAE Method to Various Commercial Samples

Utilizing the optimized MAE method, extracts were obtained from a range of distinct ginger samples. The outcomes are presented in Table 6, revealing that the concentrations of gingerols and shogaols derived from the ginger samples fell within the range of 7.58–15.84 mg Y_{TGSC} g⁻¹. Sample 8 produced the highest concentration of Y_{TGSC}. 6-gingerol was the compound obtained at the highest concentrations, except in sample 7, where its analogue, 6-shogaol, was the one found in higher proportions. Other authors have reported that the content of this compound may vary according to the drying temperature and the extraction conditions [44]. The differences observed between the Y_{TGSC} concentrations of the different samples analyzed could be explained by a series of factors such as the location of origin, harvest time, climate, soil type, variety, or drying and extraction method [45,46]. Finally, the data obtained in this research coincide with those presented by González et al., 2023 [21], where shogaols and gingerols were also extracted from ginger samples, but where ultrasound-assisted extraction instead of MAE was applied as the extraction technique.

### Table 6. Concentrations of total and individual gingerol and shogaol compounds (mg g⁻¹ sample) obtained by optimal MAE conditions in different commercial samples (n = 3).

<table>
<thead>
<tr>
<th>Commercial Samples</th>
<th>6-G ± 100 a</th>
<th>6-S ± 100 b</th>
<th>8-G ± 100 c</th>
<th>8-S ± 100 d</th>
<th>10-G ± 100 e</th>
<th>10-S ± 100 f</th>
<th>Y_{TGSC} ± 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>4.36 ± 0.30</td>
<td>1.34 ± 0.09</td>
<td>1.75 ± 0.15</td>
<td>0.88 ± 0.29</td>
<td>0.47 ± 0.32</td>
<td>0.65 ± 0.05</td>
<td>9.48 ± 0.89</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2.65 ± 0.51</td>
<td>0.88 ± 0.12</td>
<td>3.00 ± 0.65</td>
<td>0.54 ± 0.19</td>
<td>0.31 ± 0.22</td>
<td>0.17 ± 0.02</td>
<td>7.58 ± 1.52</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4.95 ± 0.54</td>
<td>1.55 ± 0.15</td>
<td>2.69 ± 0.21</td>
<td>1.20 ± 0.12</td>
<td>0.45 ± 0.16</td>
<td>0.34 ± 0.29</td>
<td>14.31 ± 1.33</td>
</tr>
<tr>
<td>Sample 4</td>
<td>4.72 ± 0.07</td>
<td>1.59 ± 0.33</td>
<td>1.51 ± 0.07</td>
<td>1.04 ± 0.35</td>
<td>0.62 ± 0.37</td>
<td>0.92 ± 0.72</td>
<td>10.43 ± 1.41</td>
</tr>
<tr>
<td>Sample 5</td>
<td>4.79 ± 1.10</td>
<td>1.64 ± 0.19</td>
<td>1.69 ± 0.68</td>
<td>1.18 ± 0.06</td>
<td>0.47 ± 0.12</td>
<td>2.20 ± 1.26</td>
<td>12.00 ± 3.3</td>
</tr>
<tr>
<td>Sample 6</td>
<td>6.24 ± 0.10</td>
<td>2.10 ± 0.10</td>
<td>1.98 ± 0.61</td>
<td>1.23 ± 0.04</td>
<td>0.72 ± 0.04</td>
<td>3.39 ± 0.06</td>
<td>15.67 ± 0.58</td>
</tr>
<tr>
<td>Sample 7</td>
<td>3.33 ± 0.05</td>
<td>6.52 ± 0.13</td>
<td>0.96 ± 0.01</td>
<td>0.66 ± 0.00</td>
<td>0.55 ± 0.09</td>
<td>0.32 ± 0.13</td>
<td>12.37 ± 0.06</td>
</tr>
<tr>
<td>Sample 8</td>
<td>6.28 ± 0.03</td>
<td>2.01 ± 0.21</td>
<td>1.53 ± 0.06</td>
<td>1.38 ± 0.10</td>
<td>0.84 ± 0.18</td>
<td>3.78 ± 0.03</td>
<td>15.84 ± 0.23</td>
</tr>
</tbody>
</table>

Different letters in the same column denote statistically significant differences as determined by Tukey’s test at a significance level of 95%.

### 4. Conclusions

In this study, an optimization approach employing BBD in conjunction with RSM was employed to optimize a MAE method for extracting the bioactive compounds, namely gingerols and shogaols, from ginger rhizomes. The optimal conditions for extraction were determined as follows: 87% ethanol in water as the extraction solvent, an extraction temperature of 100 °C, and a sample-to-solvent ratio of 0.431 g:20 mL. The extraction time of 5 min was found to be optimal, and microwaves were applied at a power of 800 W. The developed MAE method underwent successful validation for repeatability and intermediate precision, with CVs below 5%. Subsequently, the optimized method was applied to various samples obtained from different markets, illustrating its adaptability to diverse chemical compositions. Based on our findings, the developed MAE method enables the efficient extraction of gingerols and shogaols in a time-saving manner, thereby offering cost and time benefits. This fast and effective method can be used both by industries to obtain extracts enriched in gingerols and shogaols and by control and analysis laboratories to determine the quality of ginger and products made from ginger. In addition, the possibility of evaluating improvements in extraction through MAE remains open, such as MAE water extraction combined with steam explosion to obtain extracts free of organic solvents or ultrasound-assisted MAE, a combination of both techniques that could improve extraction times using lower temperatures.

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