Kinetic study on polyphenol and antioxidant activity from karonda fruit (Carissa carandas) extraction via microwave

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Abstract. Karonda fruit (Carissa carandas L.) is a tropical fruit rich in biological value due to its high total polyphenol content with potential antioxidant activity. In this study, the kinetics of total polyphenol and antioxidant activity extraction from the karonda fruit by microwave-assisted extraction technique was performed. Microwave power varies from 150, 300, and 450 (W); the ratio of material/solvent varies from 1:20, 1:40, and 1:60 (g/mL). The results show that changes in total polyphenol concentration and antioxidant activity during extraction can be predicted by a second-order kinetic model. Increasing the microwave power or amount of extraction solvent had the effect of increasing the extraction efficiency and extraction rate constant. However, the saturation concentration and the initial extraction rate increased when MW power increased or the amount of extraction decreased. In further study, the highest quality of extract, collected at the optimized conditions of microwave-assisted extraction, could be used to develop new food products for the health benefits of customers.

1. Introduction
Carissa carandas L. is a tropical plant in the Apocynaceae family. The tree is native to India and is grown in Taiwan, Indonesia, Malaysia, Myanmar, Sri Lanka, Thailand, and the Pacific Islands [1]. All parts of C. carandas are medicinal, so they are often used in traditional remedies to treat the disease. The fruit of the tree is of particular interest in published studies. Not only can the unripe fruit be used to treat liver dysfunction and lower fever, they have also been studied for their anti-diabetic, analgesic, and anti-inflammatory properties [2], [3]. The ripe fruit is used as a substance to cure vitamin C deficiency and a remedy for irritability. Karonda fruits have a characteristic red color thanks to the anthocyanin content (belonging to the polyphenol group). The polyphenol component has been reported to be the most important for human health [4]. Although our bodies have antioxidants, supplementing with antioxidants in the diet has been reported to protect against oxidative damage [5]. Phenolic compounds, the largest and most diverse group of secondary metabolites in plants, are effective antioxidants [6]. Currently, research related to karonda, as well as its biologically active components such as polyphenol compounds and antioxidant activity, are limited.

Extraction is an essential technique in the field of the food industry. There are many proposed extraction methods, especially techniques with the support of other processes to increase extraction efficiency, reduce time, or increase the ability to extract selectively. The microwave-assisted extraction technique (MAE) is considered to be a new technique for obtaining solutes using microwave energy [7]. Under the penetration effect of the microwave, electromagnetic energy is converted into heat generated in the raw materials by polar molecular rotation and ion conduction [8]. MAE is also recognized as a green technology because it reduces the use of organic solvents [9]. Microwave has the effect of volumetric heating, thus reducing the heating time and reducing the thermal gradients. Compared with the traditional extraction method, MAE can extract natural compounds with higher speed and recovery...
such as the extraction of polyphenols and caffeine from green tea [10]; ginsenosides from ginseng root [11]; E- và Z-guggolsterone, cinnamaldehyde and tannin from the medicinal Asian plants [12]; flavonoids and phenolics from Chinese quince [13]. MAE was shown as an appropriate technique for the extraction of organic and organometallic compounds because they require structural integrity when obtained. In the field of extraction operation, the kinetic extraction models played an essential role in the prediction of extraction process characteristic to design experiments, optimize extraction conditions as well as scale-up of the process [14]. Kinetics study of conventional and assisted batch solvent extraction was comprehensively summarized by Chan et al. [14]. The previous studies mainly used Fick’s law, rate law, or empirical models to analyze kinetic extraction of oil, bioactive compounds such anthocyanin, polyphenol, flavoinoids, etc [14]. However, studies on MAE kinetics of bioactive compounds from Karonda fruits that have not been published yet.

Therefore, in this study, MAE kinetics of the polyphenol content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity from karonda fruit were investigated. Two important factors of the MAE technique, including microwave power (MW) and material/solvent ratio, were surveyed. Experimental data were used to select an appropriate extraction model that predicted changes in polyphenol content and DPPH free radical scavenging activity during the MAE. Kinetic parameters were determined and analyzed. The results of this study were of great significance in the experimental design and optimization of the MAE process to achieve technological goals.

2. Materials and methods

2.1. Materials and chemicals
Karonda fruits (Carissa carandas L.) were purchased from the garden located in An Phu Dong Ward, District 12, Ho Chi Minh City. Fruits used in the study were in the ripening stage with black purple fruits, size from 2–3 cm, weight from 1–2 g. Raw materials after receiving were washed, frozen at -18°C. Before extraction, samples were pretreated by thawing, blanched at 80°C, cooled quickly with cold water, drained, seeded, and then cut into small pieces.
Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich. All the other reagents used for analyses were of analytical grade.

2.2. MAE process
The karonda fruits were extracted with distilled water. The design of the experiment used was full factorial design with two factors, including the MW power (150, 300, and 450 W) and the ratio of materials to solvent (1:20, 1:40, and 1:60 g/mL). The extract was analyzed for total polyphenol content and DPPH free radical scavenging activity. The extraction process was observed during the first 15 minutes of extraction.

2.3. Analytical methods
The total polyphenol content of the extract was determined by the Folin-Ciocalteu colorimetric method (ISO 14502-1:2005). The sample solution (0.6 mL) was added to 1.5 mL of Folin-Ciocalteu reagent diluted 10 times and incubated for 5 minutes at room temperature. 1.2 mL of 7.5% Na2CO3 was added to each analytical test tube, mixed well, and then incubated at room temperature for about 60 minutes. The absorbance values of the reaction mixture were measured by UV-Vis spectrophotometer at 765 nm. The antioxidant activity of the extract was determined using DPPH free radical scavenging capacity [15]. The DPPH solution was prepared by dissolving 24 mg DPPH with 100 mL of methanol, and then stored at 4°C for 48 hours. The reagent solution was diluted with methanol to the absorbance of 1.10 ± 0.02 units at 515 nm using a spectrophotometer. The analytical solution (150 μL) was reacted with 2850 μL of the DPPH solution for 30 minutes in the dark condition. The absorbance was then taken at 515 nm using the spectrophotometer.

2.4. Identification of the chemical composition
Many mathematical theories have been used to model extraction processes. The established models are based on both theory and experiment. Many models have been applied and extensively studied for extraction curves, including Fick’s law of diffusion, chemical kinetic equations, and experimental
equations. In this study, to characterize the extraction process of TPC from karonda using the MAE technique, the second-order reaction model (referred to as the second-order model) and Fick's exponential model (referred to as exponential model) were analyzed to evaluate the compatibility with experimental data.

Second-order model [16]-[19]:

$$C = \frac{C_\infty k_1 t}{1 + C_\infty k_1 t}$$  \hspace{1cm} (1)

Where, $C_\infty$ is the saturated anthocyanin concentration (mg/mL), $k_1$ is the extraction rate constant (mL/mg/min).

Exponential model [20]:

$$\frac{C}{C_\infty} = 1 - A \exp(-kt)$$  \hspace{1cm} (2)

Where $A$ is the model constant, and $k$ is the diffusion rate constant (1/min).

The model was compared based on statistic parameters, including the lowest RMSE standard deviation and $\chi^2$ value, and the highest $R^2$ value, which were selected to choose the appropriate model [21]:

$$R^2 = 1 - \frac{\sum (C_{\text{exp}} - C_{\text{pre}})^2}{\sum (C_{\text{exp}} - C_\infty)^2}$$  \hspace{1cm} (3)

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum (C_{\text{exp}} - C_{\text{pre}})^2}$$  \hspace{1cm} (4)

$$\chi^2 = \frac{\sum (C_{\text{exp}} - C_{\text{pre}})^2}{N - Z}$$  \hspace{1cm} (5)

2.5. Data analysis
All experiments were conducted in triplicate. Values were calculated using Microsoft Excel (2016) and expressed as mean and standard deviation. MATLAB R2014 software was used to test empirical data with models based on the Levenberg-Marquardt method.

3. Results and discussions

3.1. Mathematic models of MAE process
The changes in total polyphenol content and DPPH radical scavenging activity during the extraction process under different conditions are shown in Figure 1 and Figure 2.
From the results of Figure 1 and Figure 2, the TPC extraction curves and antioxidant activity in the study were consistent with the ideal 2-phase extraction curves of plant compounds. The initial phase of the MAE process is the rapid extraction stage of TPC and its antioxidant activity, which takes place during the first 2 minutes of extraction. In this stage, the solvents on the surface of the material are quickly dissolved into the solvent. The second phase is the diffusion step, during which the rate of TPC concentration increases and the antioxidant activity decreases significantly compared to the first stage, and after a long time, the solid-liquid system will reach saturation state [22].

To study the characteristics of TPC extraction process and antioxidant activity from karonda fruits by MAE technique, the second-order model and exponential model were analyzed and evaluated for...
compatibility with experimental data. The model was compared based on statistics, including the lowest RMSE standard deviation and $\chi^2$ values, and the highest $R^2$ value to choose which model was appropriate. The appropriate model will be selected to analyze the impact of the investigated factors on the desired responses. The results of the nonlinear regression analysis of mathematical models are presented in Table 1.

Table 1. Nonlinear regression analysis of mathematical models in both TPC and DPPH radical scavenging activity extraction kinetics by MAE technique.

| Material/ solvent ratio | MW power | Second-order | Exponential |
|-------------------------|----------|--------------|-------------|
|                         | R$^2$    | RMSE         | $\chi^2$    | R$^2$ | RMSE | $\chi^2$ |
| **TPC**                |         |              |             |       |      |          |
| 1:20                    | 150 W    | 0.9905       | 0.03645     | 1.41E-03 | 0.9132 | 0.04175 | 1.85E-03 |
|                         | 300 W    | 0.9958       | 0.03294     | 1.15E-03 | 0.9229 | 0.04336 | 2.00E-03 |
|                         | 450 W    | 0.9980       | 0.02528     | 6.79E-04 | 0.9311 | 0.04781 | 2.43E-03 |
| 1:40                    | 150 W    | 0.9925       | 0.01578     | 2.64E-04 | 0.8729 | 0.02798 | 8.32E-04 |
|                         | 300 W    | 0.9950       | 0.01121     | 1.34E-04 | 0.8941 | 0.02592 | 7.14E-04 |
|                         | 450 W    | 0.9985       | 0.01062     | 1.20E-04 | 0.8680 | 0.02873 | 7.87E-04 |
| 1:60                    | 150 W    | 0.9972       | 0.00899     | 8.59E-05 | 0.8782 | 0.02808 | 8.38E-04 |
|                         | 300 W    | 0.9993       | 0.00515     | 2.82E-05 | 0.8685 | 0.02881 | 8.82E-04 |
|                         | 450 W    | 0.9991       | 0.01221     | 1.58E-04 | 0.7917 | 0.03098 | 1.02E-03 |
| **DPPH radical scavenging activity** | | | | | | | |
| 1:20                    | 150 W    | 0.9953       | 0.03823     | 1.55E-03 | 0.8238 | 0.10588 | 1.19E-02 |
|                         | 300 W    | 0.9942       | 0.04382     | 2.04E-03 | 0.9062 | 0.06689 | 4.75E-03 |
|                         | 450 W    | 0.9981       | 0.03392     | 1.22E-03 | 0.8884 | 0.07821 | 6.50E-03 |
| 1:40                    | 150 W    | 0.9985       | 0.01561     | 2.59E-04 | 0.8454 | 0.03554 | 1.34E-03 |
|                         | 300 W    | 0.9987       | 0.01609     | 2.75E-04 | 0.8568 | 0.03361 | 1.20E-03 |
|                         | 450 W    | 0.9997       | 0.00934     | 9.26E-05 | 0.8528 | 0.03673 | 1.43E-03 |
| 1:60                    | 150 W    | 0.9974       | 0.01506     | 2.41E-04 | 0.9246 | 0.01907 | 3.86E-04 |
|                         | 300 W    | 0.9972       | 0.01498     | 2.38E-04 | 0.9017 | 0.02136 | 4.85E-04 |
|                         | 450 W    | 0.9993       | 0.01358     | 1.96E-04 | 0.8530 | 0.02989 | 9.43E-04 |

Based on the results of Table 1, the second-order model has a very high correlation coefficient; all are greater than 0.99. Meanwhile, the exponential model has a relatively low correlation coefficient, ranging from 0.7027 to 0.9311. In addition, the statistic values such as RMSE and $\chi^2$ of the second-order model are lower than the exponential model. Specifically, the second-order model has RMSE and $\chi^2$ in the range of 0.00515–0.03645 and 2.82E–05–1.41E–03, respectively; the exponential model has RMSE and $\chi^2$ between 0.02538–0.05599 and 6.84E–04–3.33E–03, respectively. When comparing each treatment condition, the second-order model has a higher $R^2$ value and lower RMSE and $\chi^2$ values than the exponential model. Thus, in this study, when changing MW power and material/solvent ratio, the second-order model predicted changes in TPC content during the extraction of karonda fruit better than the exponential model. Many studies have identified suitable second-order models when studying extraction of polyphenols from carob (Ceratonia siliqua L.) using UAE techniques [23]; grape cane by CE technique [24]; grape seeds by HVEDE [25] or CE [26], soybeans by CE [27], pomegranate peel by UAE [16] and CE [17].

Similar to TPC extraction process, the results of Table 1 also show that the second-order model has better predictive power than the exponential model with the entire correlation coefficient value greater than 0.99, and statistical parameters for error estimation are smaller than the exponential model. Several studies on antioxidant extracts have applied the second-order model for extraction, such as extraction of pomegranate marc by CE technique [17], and pomegranate peels by UAE technique [16].
3.2. Effect of MW power and material/solvent ratio on TPC extraction

From the second-order kinetic model, the parameters for MAE process for karonda fruits were determined including saturated anthocyanin concentration \( C_s, \text{mg/mL, with } C_s = C_o \), saturated extraction efficiency \( Y_s, \text{mg/g, with } Y_s = \frac{C_s \times V}{m} \), where \( V \) is the total extraction volume, \( m \) is the sample mass), extraction rate constant \( k, \text{mL/mg/min} \), initial extraction rate \( h, \text{mg/mL/min, with } h = k_s C_o^2 \). The results are shown in Table 2.

Table 2. Kinetic parameters of TPC extraction process from karonda by MAE technique.

| Material/solvent ratio | MW power | \( C_s \) (mgGAE/mL) | \( Y_s \) (mgGAE/g) | \( k \) (mL/mgGAE/min) | \( h \) (mgGAE/mL/min) |
|------------------------|----------|----------------------|---------------------|------------------------|-----------------------|
| 1:20                   | 150 W    | 0.66723              | 13.34               | 1.38149                | 0.61503               |
|                        | 300 W    | 0.72341              | 14.47               | 1.45866                | 0.76334               |
|                        | 450 W    | 0.76064              | 15.21               | 1.63037                | 0.94329               |
| 1:40                   | 150 W    | 0.30944              | 12.38               | 3.39128                | 0.32472               |
|                        | 300 W    | 0.33055              | 13.22               | 3.47662                | 0.37986               |
|                        | 450 W    | 0.36501              | 14.60               | 3.87704                | 0.51653               |
| 1:60                   | 150 W    | 0.29144              | 17.49               | 3.14592                | 0.26721               |
|                        | 300 W    | 0.31628              | 18.98               | 3.99809                | 0.39995               |
|                        | 450 W    | 0.33178              | 19.91               | 4.63300                | 0.51000               |

The ratio of material/solvent is very important in MAE technique because it is necessary to ensure that the material is completely immersed in the extraction solvent and ensure a uniform sample heating rate. The material/solvent ratio investigated in this study ensured complete immersion of the test sample in the surveyed solvent. The ratio of material/solvent can be changed by keeping the solvent volume unchanged (changing the sample weight) or keeping the volume constant (changing the solvent volume). In this study, the volume of the solvent was kept constant, and the volume of the material was changed. Therefore, at the same MW power level, when changing the material/solvent ratio, the energy absorbed by the solvent was considered to be not significantly different. From the results of Table 2, it is shown that when changing the ratio of materials/solvents from 1:20 to 1:60, the extraction efficiency increases. At low solvent ratios, mass transfer barriers affected the diffusion of compounds from plant cells, resulting in low extraction efficiency [17], [28]. Increasing the amount of solvent will increase the concentration gradient, which will promote mass transfer and increase extraction efficiency [17]. In other words, a higher amount of solvents will increase the solubility of the solute. According to previous studies, when the material/solvent ratio decreased, the extraction efficiency of phenolic compounds increased, until the optimal value was reached [29]. For saturated TPC concentration, the results show that when changing the ratio of materials/solvents from 1:20 to 1:60 at the same MW power, the lower TPC saturated concentration was obtained. The result was consistent with the extraction principle for solid-liquid systems [30]. Although the TPC saturated concentration was lower, the saturated extraction efficiency was still higher when the extraction at a 1:60 ratio. Thus, the use of more solvents will improve the efficiency of the extraction process. The results were similar to the conclusions in the research of Krishnan and Rajan [31]. At the same MW power, the initial extraction rate decreased when
the material/solvent ratio changed from 1:20 to 1:60, possibly due to the lower saturated concentration at 1:60. The initial extraction rate \( h \), mg/mL/min) was calculated by following equation \( h = k_i C^2 \); therefore the initial extraction rate was proportional to the extraction rate constant \( (k_i, \text{mL/mg/min}) \) and the square of the saturated concentration \( C^\infty, \text{mg/mL} \) [32]. This showed that the saturated concentration affected the initial extraction rate greater than the extraction rate constant.

The specification of the extraction process of TPC from karonda with MAE technique also changed with the change of MW power. Corresponding to each material/solvent ratio, the saturated extraction efficiency increased from 13.34 to 15.21 (at a 1:20 ratio), from 12.38 to 14.60 (at a 1:40 ratio), from 17.49 to 19.91 (at a 1:60 ratio) when MW power increased from 150 W to 450 W. The influence of MW on extraction process occurred in both the beginning and the second stages of the extraction process. At the beginning of the extraction process, the increase in radiation power caused the amount of water in the solid-liquid system to absorb more energy and the temperature increased rapidly thanks to the generation of volumetric heating through the ion conduction and molecular rotation mechanism [33], leading to a possible acceleration of the solvent-solid interaction. As the temperature increased, the molecular motion and the coefficient of diffusion increased significantly. Besides, solvents at high temperatures also had higher solubility. The continuous movement of molecules together with the increase in the interaction between solvents-solids increased friction between solids and solvents. This contributes to the elimination of solutes from the solid surface [34]. In the second stage of extraction, corresponding to the internal diffusion process, this stage had a lower rate than the external diffusion process. MW power accelerated the movement of molecules and internal diffusion, and disrupted plant cell structure [35]. According to Dong et al. [34], the broken networks become soft and allow many solvents to diffuse into the plant cell network. MW power causes physical damage such as scorching, over-heating, charring, and even temperature distribution [36]. When the cells were destroyed, the volume increased by increasing solubility and improving the contact between solutes and solvents [31]. The greater the degree of disruption, the higher the solubility concentration obtained. However, Gao et al. [37] also confirmed that when applying higher MW power, it is difficult to control extraction temperature. For saturated concentration, at the same the material/solvent ratio, when increasing MW power from 150 W to 450 W, the value of saturated concentration also increased from 0.66723 to 0.76064 (at a 1:20 ratio), from 0.30944 to 0.56501 (at a 1:40 ratio), from 0.29144 to 0.33178 (at a 1:60 ratio). Increasing the saturated concentration at a higher power was because the higher MW power weakened the interactions between solute-solute and solute-solid [31]. The extraction rate constant also increased with increasing MW capacity, possibly due to higher heat energy which increased solubility. Initial extraction rates were also higher at higher MW capacities.

3.3. Effect of MW power and material/solvent ratio on antioxidant activity extraction

From the second-order kinetic model, the parameters of MAE process were determined including saturated antioxidant capacity (Cs, mg TE/mL), saturated extraction efficiency (Ys, mg TE/g), extraction rate constant (k, mL/mg TE/min), initial extraction rate (h, mg TE/mL/min). The results are detailed in Table 3.
Table 3. Kinetic parameters of the DPPH free radical scavenging extraction process from karonda by MAE technique.

| Material/solvent ratio | MW power | C_s (mgTE/mL) | Y_s (mgTE/g) | k (mL/mgTE/min) | h (mgTE/mL/min) |
|------------------------|----------|---------------|--------------|----------------|-----------------|
| 1:20                   | 150 W    | 0.97812       | 19.56        | 0.87797        | 0.83997         |
| 300 W                  | 1.05758  | 19.15         | 1.02044      | 1.14133        |
| 450 W                  | 0.99868  | 19.97         | 0.91607      | 0.91365        |
| 1:40                   | 150 W    | 0.45855       | 18.34        | 3.27062        | 0.68772         |
| 300 W                  | 0.46919  | 18.77         | 3.40984      | 0.75063        |
| 450 W                  | 0.48349  | 19.34         | 4.27145      | 0.99851        |
| 1:60                   | 150 W    | 0.34771       | 20.86        | 3.38733        | 0.40954         |
| 300 W                  | 0.35367  | 21.22         | 4.00182      | 0.50055        |
| 450 W                  | 0.42767  | 25.66         | 4.70847      | 0.86119        |

In this study, it was found that when the material/solvent ratio changed from 1:20 to 1:60 at the same MW power, the extraction rate constant increased significantly, but the saturated concentration and initial extraction rate decreased. For the extraction efficiency, the changes were unclearly when the material/solvent ratio changed from 1:20 to 1:40 at the same MW power; however, it was increased significantly when the material/solvent ratio changed from 1:20 to 1:60. At the same material/solvent ratio, when increasing the MW, the specific extraction parameters include saturated concentration, saturated extraction efficiency, extraction rate constant, and initial extraction rate increased. Antioxidant activity in karonda fruit extract was mainly affected by phenolic compounds, especially anthocyanins and flavonoids. Therefore, in this study, the mechanism of the effects of the ratio of materials/solvents and MW power on the extraction of antioxidant activity follows the same rule as the TPC extraction process.

4. Conclusions
The kinetics of extraction of TPC and the antioxidant activity from karonda fruit using the MAE technique was performed and evaluated. The results show that the second-order model was suitable for predicting changes in TPC concentration and antioxidant activity during the MAE process. The MW power and the material/solvent ratio significantly affected the MAE process. The increase in MW power had the effect of increasing TPC concentration and antioxidant activity, extraction efficiency, extraction rate constant, and initial extraction rate in saturation state thanks to the ability of MW energy to increase molecular movement, cell disruption and consequent increase in extraction efficiency. Meanwhile, at the same MWPD, the change of the material/solvent ratio from 1:20 to 1:60 had the effect of increasing extraction rate constant, but decreasing the saturated concentration and initial extraction rate. For further study, the optimization of MEA conditions should be conducted to obtain the highest quality of extract. This extract could be used to develop new food products for the health benefits of customers.

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