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Morinda citrifolia L. (Noni) fruits: Optimising extraction kinetics of yield and flavonoids for enhanced antioxidant activity at different temperatures

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Abstract

Morinda citrifolia L., commonly known as 'Noni', holds significant potential in pharmaceutical research due to its bioactive constituents. This study aimed to optimise the ultrasound-assisted extraction process for yield and total flavonoid content (TFC) from *M. citrifolia* fruits at different temperatures (40 °C, 50 °C, and 60 °C). The extraction kinetics were analysed using four models: first-order kinetic, Peleg, power law, and two-site kinetic, determined through goodness-of-fit. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Results showed the peak extraction yield at 40 °C within 90 min, yielding 1.46 ± 0.01 mg/mL, while the peak TFC was at 50 °C within 100 min, yielding 3.43 ± 0.00 mg CE/g db. The two-site kinetic model best described the extraction kinetics based on goodness of fit. The IC_{50} value for DPPH inhibition was 1883.57 ± 0.02 µg/mL at 50 °C, indicating moderate antioxidant activity compared to ascorbic acid (95.11 ± 0.13 µg/mL). Overall, *M. citrifolia* fruits provide valuable insights for standardised extraction methods, potentially benefiting therapeutic applications and indirectly reducing operational costs and time for manufacturers.

Keywords: *Morinda citrifolia*, Yield, Flavonoids, Extraction kinetics, Antioxidant

1. Introduction

Scientific researchers have lately shown great interest in *Morinda citrifolia* L., commonly known as 'Noni', because of its many benefits [1, 2]. The *M. citrifolia* plant, which is native to Southeast Asia, spread to Australia, Hawaii, the French Polynesian Islands, and other tropical areas—possibly through hydrochory by buoyant seed dispersal or transportation by early migrants or explorers [1]. This attribute explains its widespread presence in the Indo-Pacific Islands, aided by the migratory patterns of seafaring Polynesians. Scientific research has explored various parts of *M. citrifolia*, encompassing fruits, roots, leaves, and seeds, all of which find applications in both culinary and medicinal contexts [2]. Abou Assi et al. [3] comprehensively listed various pharmacological effects provided by *M. citrifolia*, including antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, antitumour, hypotensive, anthelminthic, and immune-enhancing effects. They have also been commercially available in the United States of America (USA) since the 1990s. Additionally, the European Commission granted approval for the fruit juice of *M. citrifolia* to be designated as a novel food in 2010 [4]. This official announcement shows that *M. citrifolia* juice has great potential as a new food product.

Flavonoids are a class of natural substances with mixed phenolic structures, sourced from various things, including fruits, vegetables, tea, wine, bark, flowers, grains roots, and stems [5]. In the case of *M. citrifolia*, it has been observed that its root extracts contain bioactive compounds such as flavonoids, coumarins, and anthraquinones. These compounds exhibit a range of biological activities, including antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral effects [6]. Crude extracts from different plant parts and fruit juice are documented to contain a variety of compounds, including flavonoids, anthraquinones, terpenoids, iridoids, sterols, amino acids, fatty acids, lignans, polysaccharides, and sugars [7]. A comprehensive review on *M. citrifolia* also highlights the presence of flavonoids among other biochemically active compounds in this plant [2].

The dynamic behaviour of phytochemical extraction from plants is understood in many scientific studies through the use of extraction kinetics. This technique involves mathematical models such as first-order kinetic, Peleg, power law, and two-site kinetic, which are commonly used for solute recovery from solid materials. These models play a significant role in fitting experimental data and visualising the process through graph plotting. The first-order kinetic model, often used to describe chemical reaction rates, assumes the rate is directly proportional to the reactant concentration and has been a model for substrate utilisation in complex waste for many

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years [8]. The Peleg model explains sorption phenomena for food systems in food science and engineering, positing that the rate of sorption depends on both initial and equilibrium moisture contents, effectively describing some experimental sorption data [9]. In plant phytochemical extraction, the power law model refers to a mathematical relationship between extraction kinetics and phytochemical concentration in plant extracts, suggesting that the rate of extraction increases with the concentration raised to some power [10]. Furthermore, in the field of adsorption, the two-site kinetic model differentiates between diffusional and surface reaction kinetic models, demonstrating its utility in elucidating the underlying mechanisms of adsorption processes [11].

Despite the potential therapeutic benefits of *M. citrifolia* fruits, there is a lack of scientific exploration and understanding of their extraction kinetics. This knowledge gap restricts the development of efficient extraction processes and standardised methods for manufacturers. High-temperature extraction methods typically applied in the field result in the loss of certain ingredients from the fruits, making them uneconomical for use. Additionally, the lack of global awareness about the health benefits of *M. citrifolia* fruits has further limited their consumption. The absence of research on extraction kinetics presents a challenge in developing standardised methods for manufacturers, thereby increasing operational costs and time consumption. The employment of mathematical modelling as a powerful engineering tool facilitates the systematic analysis of the extraction process and predicts system behaviour under different conditions. Hence, this study aims to investigate the extraction kinetics of yield and total flavonoid content (TFC), and the antioxidant potential of *M. citrifolia* fruits at different temperatures (40 °C, 50 °C, and 60 °C). Understanding these properties of *M. citrifolia* fruits can help bridge the knowledge gap, leading to the development of standardised extraction methods.

2. Materials and methods

2.1 Chemicals and reagents

Absolute ethanol (99.8%), sourced from Merck (Darmstadt, Germany), was used as the solvent for the extraction process. Ascorbic acid, aluminium chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and methanol, all of analytical grade, were supplied by Sigma-Aldrich (Burlington, MA, USA). These chemicals were used as received, ensuring their high purity.

2.2 Sample procurement

A batch of 2 kg of fully mature and ripe *M. citrifolia* fruits was procured from Universiti Malaysia Sabah in Sabah, Malaysia (Coordinates: 6°02'16.4" N 116°07'38.8" E). The fruits were thoroughly rinsed with tap water to remove any foreign particles from their surface. The seeds were then extracted, and the pulp was hand-cut using a stainless-steel knife. The initial moisture content of the samples were about 88%. The convection drying oven (ED 23, Binder, Neckarsulm, Germany) was pre-set to a drying temperature of 60 °C, and the samples were dried until a consistent weight was achieved. Once dried, the samples were finely ground into a powder using a grinder (EBM-9182, Elba, Borso del Grappa, Italy). The ground powder was then sieved using a test sieve with a mesh size of < 250 µm (Endecotts, London, UK). Finally, the samples were carefully sealed for subsequent analysis.

2.3 Experimental design

In this experiment, 10 g of the ground samples were weighed and mixed with absolute ethanol in a conical flask at a ratio of 1:20 g/mL (w/v) for the extraction process [12]. The extraction was initiated using ultrasound-assisted extraction with a sonication water bath (CPX8800H, Branson, Brookfield, CT, USA) set at a frequency of 37 kHz and 70 W power input. The process was conducted at 40 °C, with intermittent swirling every 10 min over a 120-minute period. This procedure was repeated at two additional temperatures, 50 °C and 60 °C. The samples were then filtered using Whatman No. 1 filter paper (Maidstone, Kent, UK). The resulting filtrate supernatants were processed using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany) until crude extracts were obtained. These crude extracts were further dried in a convection drying oven to remove any remaining solvent.

2.4 Extraction yield

The extraction yield of the crude extract was evaluated following the procedure outlined by Lin et al. [13] with minor modifications. The evaporated dried extract was weighed using a digital balance (ADB 100-4, Kern & Sohn GmbH, Balingen, Germany). The concentration-based yield was determined using Eq. (1):

$$C_a = \frac{m}{V} \quad (1)$$

where C_a is the concentration of crude extract (mg/mL), m is the weight of dried extract (mg), and V is the volume of the solvent used for the extraction (mL).

2.5 Total flavonoid content

The TFC in the crude extract was determined using the method outlined by Awang et al. [14] and Hobbi et al. [15] with slight adjustments, employing an aluminium chloride colorimetric assay. Initially, 1 mL of crude extract was blended with 1 mL of 10% aluminium chloride, and the resulting mixture was allowed to incubate in the dark for 15 min at room temperature. The absorbance of the solution was then measured at 510 nm using a UV-Vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA). TFC was quantified in mg CE/g db, employing catechin as the standard reference based on the calibration curve ($y = 0.0589x - 0.0277$). Eq. (2) was employed to measure the TFC in the crude extract:

$$\text{Concentration of TFC (mg CE/g db)} = \frac{C_b \times V}{m} \quad (2)$$

where C_b is the concentration of crude extract (mg/mL) obtained from the standard curve of TFC.

2.6 Extraction kinetic models

2.6.1 First-order kinetic model

The first-order kinetic model as suggested by Rakshit et al. [16], was employed, as the mass equilibrium of yield or TFC for a unit liquid volume during extraction can be expressed by the following Eq. (3):

$$\frac{dC_t}{dt} = -k \cdot (C_s - C_t) \quad (3)$$

By integrating Eq. (4) to determine the kinetic parameters, the following boundary conditions are applied: from $C_t = 0$ to C_t and from $t = 0$ to t, resulting in the outcome expressed by Eq. (5):

$$\int_0^{C_t} \frac{dC_t}{C_s - C_t} = -k \int_0^t dt \quad (4)$$

$$C_t = C_s \cdot (1 - e^{-k \cdot t}) \quad (5)$$

where C_t is the concentration of yield (mg/mL) or TFC (mg CE/g db) obtained from crude extract at a specific time t (min), C_s is the concentration of yield (mg/mL) or TFC (mg CE/g db) at the saturation point, and k is the first-order rate constant (min^{-1}).

2.6.2 Peleg model

The model proposed by Peleg [17] to describe sorption curves was adapted to characterise the solid-liquid extraction process according to Eq. (6):

$$C_t = C_0 + \frac{t}{k_1 + k_2 \cdot t} \quad (6)$$

where k_1 is Peleg's rate constant (relating to the extraction rate at the initial stage of the extraction process) of yield (mL·min/mg) or TFC (g db·min/mg CE), k_2 is Peleg's capacity constant (relating to the peak extraction rate throughout the extraction process) of yield (mL/mg) or TFC (g db/mg CE), and C_0 is the concentration of yield (mg/mL) or TFC (mg CE/g db) at time $t = 0$. At $t = 0$, $C_0 = 0$. Thus, Eq. (7) was modified to the following form:

$$C_t = \frac{t}{k_1 + k_2 \cdot t} \quad (7)$$

2.6.3 Power law model

The power law model has been used to describe the extraction process in solid-liquid extraction [18]. It can be represented by the following Eq. (8):

$$C_t = B \cdot t^n \quad (8)$$

where B is the constant related to the extraction rate of yield (mg/mL·min) or TFC (mg CE/g db·min), and n represents the diffusional exponent, which must be < 1 when extracting from plant cells.

2.6.4 Two-site kinetic model

The two-site kinetic model posits a two-stage extraction process involving washing and diffusion [19]. The model is expressed by the following Eq. (9):

$$C_t = C_w \cdot \left[1 - e^{(-k_w \cdot t)}\right] + C_d \cdot \left[1 - e^{(-k_d \cdot t)}\right] \quad (9)$$

where C_w and C_d are the concentration of yield (mg/mL) or TFC (mg CE/g db) recovered during the washing and diffusion stages, respectively, and k_w and k_d are the extraction rate coefficients of yield (mL/mg·min) or TFC (g db/mg CE·min) during the washing and diffusion stages, respectively.

2.7 Goodness of fits

The goodness of fit for the chosen models was compared and assessed using statistical tools, which determined the most appropriate mathematical model for both yield and TFC data. This assessment included the use of statistical metrics such as the coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), root mean square error (RMSE), chi-square (χ^2), and mean absolute error (MAE). These parameters can be mathematically represented through their corresponding statistical metrics in Eqs. (10), (11), (12), (13), and (14) as follows:

$$R^2 = 1 - \left[\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}\right] \quad (10)$$

$$R^2_{adj} = 1 - \frac{(1 - R^2) \times (n - 1)}{n - k - 1} \quad (11)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \quad (12)$$

$$\chi^2 = \sum_{i=1}^{n} \frac{(y_i - \hat{y}_i)^2}{\hat{y}_i} \quad (13)$$

$$MAE = \frac{1}{N}\sum_{i=1}^{n} |y_i - \hat{y}_i| \quad (14)$$

where k is the number of independent variables, N is the total number of data points, n is the number of observations, y_i is the observed values, $\hat{y}_i$ is the predicted values, and $\bar{y}$ is the mean of the actual values.

2.8 Antioxidant activity

The DPPH free radical scavenging assay was conducted following the procedure outlined by Stephenus et al. [12] with slight modifications. Briefly, 1 mL of the crude extract was mixed with 1 mL of a DPPH-methanolic solution (0.1 mM). The resulting mixture was incubated in the dark at room temperature for 30 min. Subsequently, the absorbance was measured spectrophotometrically at 517 nm against the blank. Ascorbic acid was used as the positive control. The free radical scavenging activity of the extract against DPPH was determined using the following Eq. (15):

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (15)$$

where A_c and A_s are the absorbances of the control and sample, respectively. The results were presented as IC_{50} (half maximal inhibitory concentration) values, derived from regression analysis, indicating the concentration required to inhibit 50% of DPPH radicals.

2.9 Statistical analysis

The experiments were conducted in triplicates, and the results are reported as mean ± standard deviation (SD). The data underwent one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$ as the level of significance, using SPSS (Version 28) as the statistical software. The extraction kinetics were modelled using Excel Solver (Microsoft Office 2019).

3. Results

3.1 Effect of temperatures on extraction kinetics of yield

The experimental results for the yield from *M. citrifolia* fruits at different temperatures (40 °C, 50 °C, and 60 °C) over a period of 0 to 120 min are detailed in Figure 1. Initially, there was a sharp rise in yield, noticeable within the first 10 to 50 min at 40 °C, 10 to 80 min at 50 °C, and 10 to 110 min at 60 °C. This was followed by a gradual and slower increase, stabilising around 60 min at 40 °C and persisting until reaching maximum yield at 90 min. Nevertheless, at 50 °C and 60 °C, the trend shows a gradual increase, with the highest yield reached between 80 to 120 min and 110 min, respectively. The data illustrates that at 40 °C, the highest yield attained within 90 min was 1.46 ± 0.01 mg/mL. In contrast, at 50 °C and 60 °C, the maximum yields achieved were 1.31 ± 0.00 mg/mL and 1.43 ± 0.00 mg/mL, respectively, both within 110 min. These findings highlight the significant impact of temperature variation on yield, as anticipated, although they do not reveal major differences in terms of resulting values. The findings also indicate an initial decrease in yield under all conditions within the first 10 min before gradually increasing.

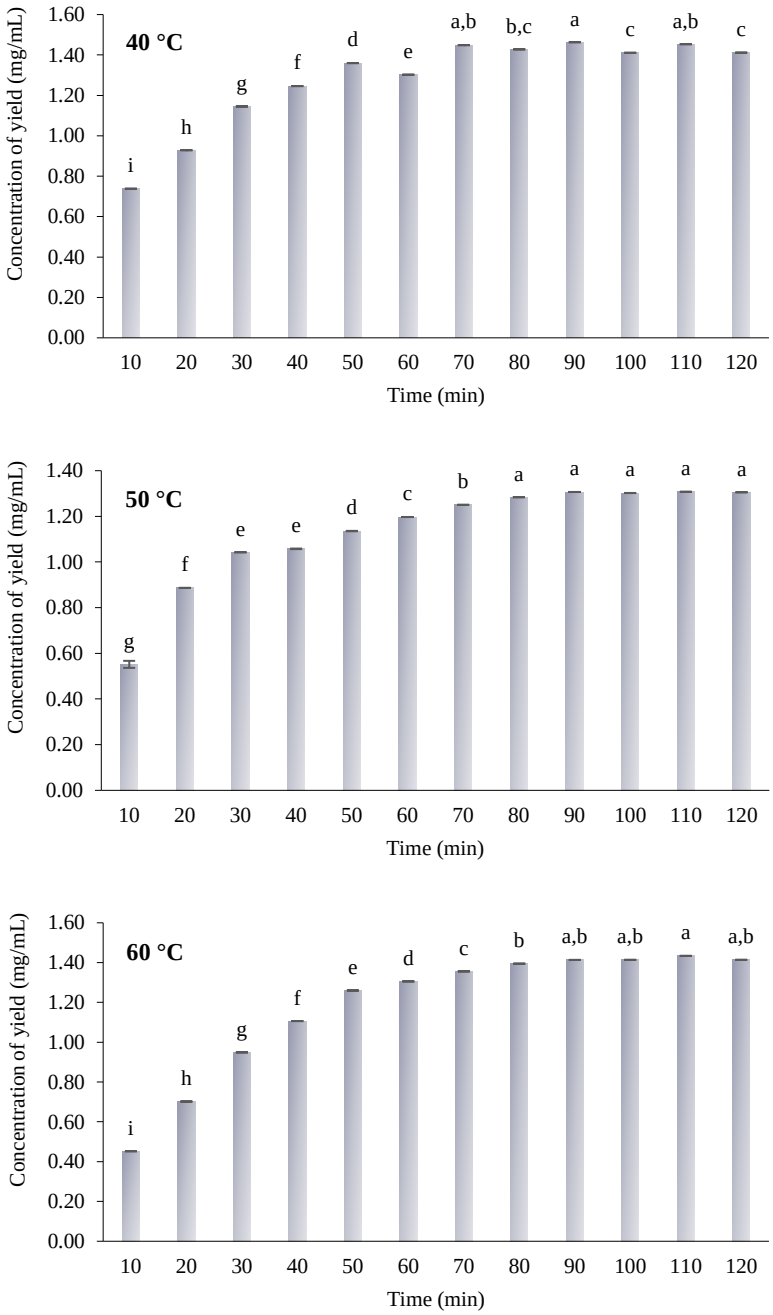

Figure 1 Concentration of yield over time of *M. citrifolia* fruits at different temperatures. Values denote mean ± SD from three replicates. Different letters (within a bar) signify statistically significant distinctions (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Four mathematical models for extraction kinetics—namely the first-order kinetic, Peleg, power law, and two-site kinetic models—were applied to characterise the extraction behaviour of yield in *M. citrifolia* fruits in this study. Figure 2 presents a comparison between the experimental and predicted extraction kinetic models of yield in *M. citrifolia* fruits at different temperatures. The model with the highest R^2 and R^2_{adj} values, along with the lowest RMSE, χ^2, and MAE values among all models were selected as the criterion for assessing the goodness of fit. Table 1 presents the results of fitting the experimental data to the extraction kinetic models of yield in *M. citrifolia* fruits at different temperatures. According to the results, the R^2 values exhibited the following variations: 0.983 to 0.990 for the first-order kinetic model, 0.989 to 0.995 for the Peleg model, 0.952 to 0.969 for the power law model, and 0.993 to 0.998 for the two-site kinetic model. This indicates strong correlations, approaching a value of 1 for all models. The R^2_{adj} values also varied: 0.082 to 0.089 for the first-order kinetic model, 0.088 to 0.095 for the Peleg model, 0.047 to 0.066 for the power law model, and 0.093 to 0.096 for the two-site kinetic model. In addition, RMSE values ranged as follows: 0.077×10^{-2} to 0.143×10^{-2} for the first-order kinetic model, 0.035×10^{-2} to 0.105×10^{-2} for the Peleg model, 0.216×10^{-2} to 0.454×10^{-2} for the power law model, and 0.024×10^{-2} to 0.059×10^{-2} for the two-site kinetic law model. Another critical parameter, χ^2, showed the lowest ranges: 0.442×10^{-4} to 14.643×10^{-4} for the first-order kinetic model, 0.000×10^{-4} to 0.039×10^{-4} for the Peleg model, 0.005×10^{-4} to 0.066×10^{-4} for the power law model, and 0.000×10^{-4} to 0.003×10^{-4} for the two-site kinetic model. MAE is also included as one of the measures of goodness of fit as follows: 0.007 to 0.042 for the first-order kinetic model, 0.000 to 0.002 for the Peleg model, 0.001 to 0.003 for the power law model, and 0.000 to 0.001 for the two-site kinetic model.

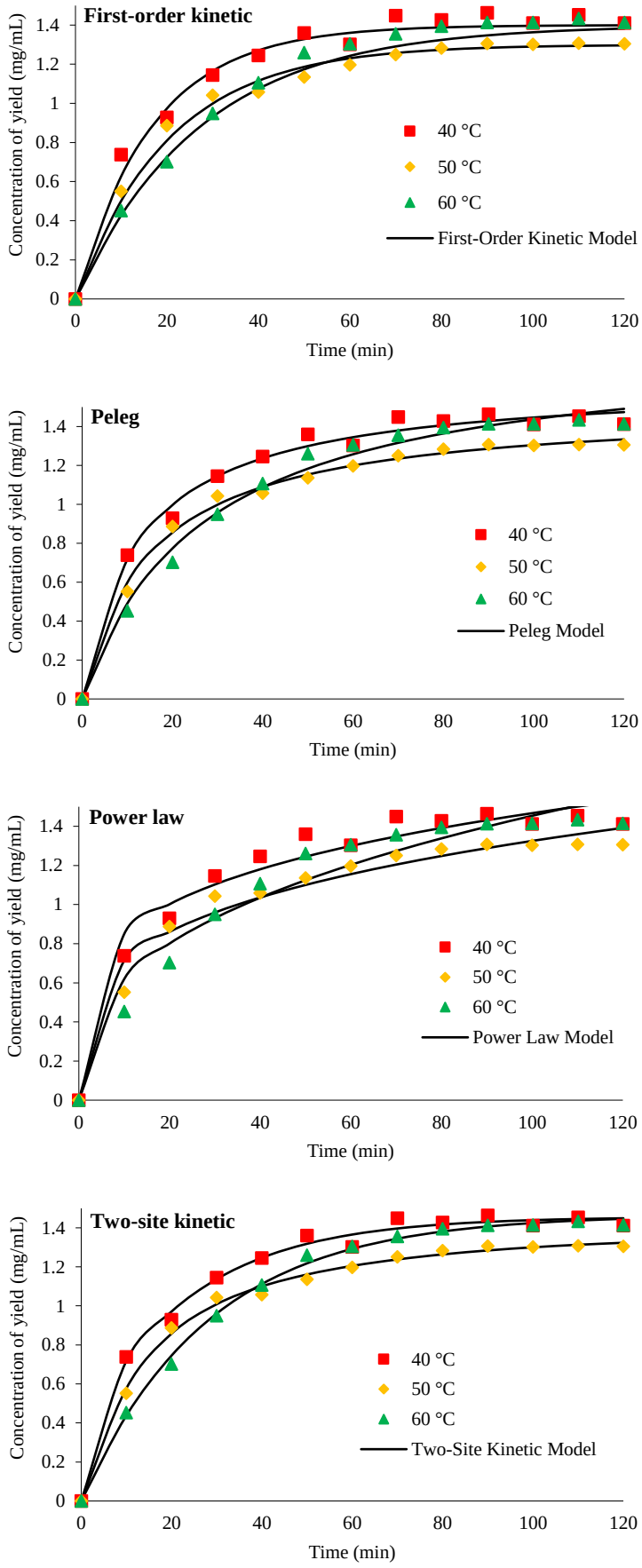

Figure 2 Comparison of experimental and predicted data for the extraction kinetic models of yield in *M. citrifolia* fruits at different temperatures.

Table 1 Goodness of fit analysis for extraction kinetic models of yield in *M. citrifolia* fruits at different temperatures.

Model	Temperature (°C)	Constant	R^2	R^2_{adj}	RMSE (10^{-2})	χ^2 (10^{-4})	MAE
First-order kinetic	40	C_e = 1.400 k = 0.059	0.983	0.082	0.143	2.819	0.020
	50	C_e = 1.300 k = 0.049	0.990	0.089	0.077	0.442	0.007
	60	C_e = 1.400 k = 0.036	0.985	0.083	0.143	14.643	0.042
Peleg	40	k_1 = 7.810 k_2 = 0.613	0.989	0.088	0.092	0.000	0.000
	50	k_1 = 10.083 k_2 = 0.666	0.995	0.095	0.035	0.004	0.001
	60	k_1 = 14.936 k_2 = 0.546	0.989	0.088	0.105	0.039	0.002
Power law	40	B = 0.492 n = 0.237	0.965	0.062	0.290	0.005	0.001
	50	B = 0.385 n = 0.269	0.969	0.066	0.216	0.010	0.001
	60	B = 0.264 n = 0.371	0.952	0.047	0.454	0.066	0.003
Two-site kinetic	40	C_w = 1.122 k_w = 0.042 C_d = 0.298 k_d = 1.880	0.993	0.093	0.059	0.000	0.000
	50	C_w = 0.597 k_w = 0.022 C_d = 1.285 k_d = 0.091	0.996	0.096	0.027	0.001	0.000
	60	C_w = 1.215 k_w = 0.035 C_d = 0.208 k_d = 0.035	0.998	0.097	0.024	0.003	0.001

Overall, the highest R^2 value was achieved for the two-site kinetic model at 50 °C (0.996), contrasting with values of 0.993 and 0.998 at 40 °C and 60 °C, respectively. This pattern is similarly reflected in the R^2_{adj} values. Additionally, the RMSE, χ^2, and MAE values for the two-site kinetic were lower than those for the other models. Based on these metrics, the two-site kinetic model demonstrated a superior fit as an extraction kinetic model for yield in *M. citrifolia* fruits at temperatures of 40 °C, 50 °C, and 60 °C. The concentrations of recovered yield (C_w and C_d) and the extraction rate coefficients (k_w and k_d) described by the two-site kinetic model significantly influence the yield and efficiency of the extraction process. C_w and C_d indicate the amount of solute recovered during the washing and diffusion stages at different temperatures, while k_w and k_d represents the rates at which the solute is removed during these stages, respectively. At 40 °C, C_w increased from 1.122 mg/mL to 1.215 mg/mL at 60 °C, suggesting that higher temperatures lead to greater concentration of yield recovered during the washing stage. Conversely, C_d varied from 0.298 mg/mL at 40 °C to 1.285 mg/mL at 50 °C, followed by a decrease to 0.208 mg/mL at 60 °C, indicating a non-linear relationship between temperature and yield concentration during the diffusion stage. The combination of k_w and k_d for different temperatures (40 °C, 50 °C, and 60 °C) provide insights into the temperature dependence of the extraction process. The observed decrease in k_w from 0.042 mL/mg·min at 40 °C to 0.022 mL/mg·min at 50 °C, followed by an increase to 0.035 mL/mg·min at 60 °C, suggests a non-linear relationship between temperature and the extraction rate during the washing stage. Similarly, k_d decreased from 1.880 mL/mg·min at 40 °C to 0.035 mL/mg·min at 60 °C, illustrating an intricate temperature-related effect on the diffusion stage extraction rate. These findings have implications for optimising the extraction process of *M. citrifolia* fruits. Understanding the temperature dependence of the extraction rate coefficients and the yield recovered can guide the design of efficient extracting processes that maximise both yield and extract quality.

3.2 Effect of temperatures on extraction kinetics of TFC

The experimental results for the TFC in *M. citrifolia* fruits at different temperatures (40 °C, 50 °C, and 60 °C) over a duration of 0 to 120 min are detailed in Figure 3. Initially, there was a sharp rise in TFC, noticeable within the first 10 to 40 min at 40 °C and 60 °C, and 10 to 50 min at 50 °C. This was followed by a gradual and slower increase, stabilising around 50 min at 40 °C and 50 °C, and persisting until reaching the maximum TFC at 100 min and 120 min, respectively. However, the trend shows a gradual decrease at 60 °C, with the highest TFC reached at 70 min, and slightly decreasing until 120 min. The data illustrates that at 50 °C, the highest TFC attained within 100 min was 3.43 ± 0.00 mg CE/g db. In contrast, under the conditions of 40 °C and 60 °C, the highest TFC values reached were 3.21 ± 0.00 mg CE/g db and 3.25 ± 0.01 mg CE/g db at 120 min and 70 min, respectively. These findings highlight the significant impact of temperature variation on TFC, as anticipated, although they do not reveal major differences in terms of resulting values. The findings also indicate an initial decrease in TFC under all conditions within the first 10 min before gradually increasing.

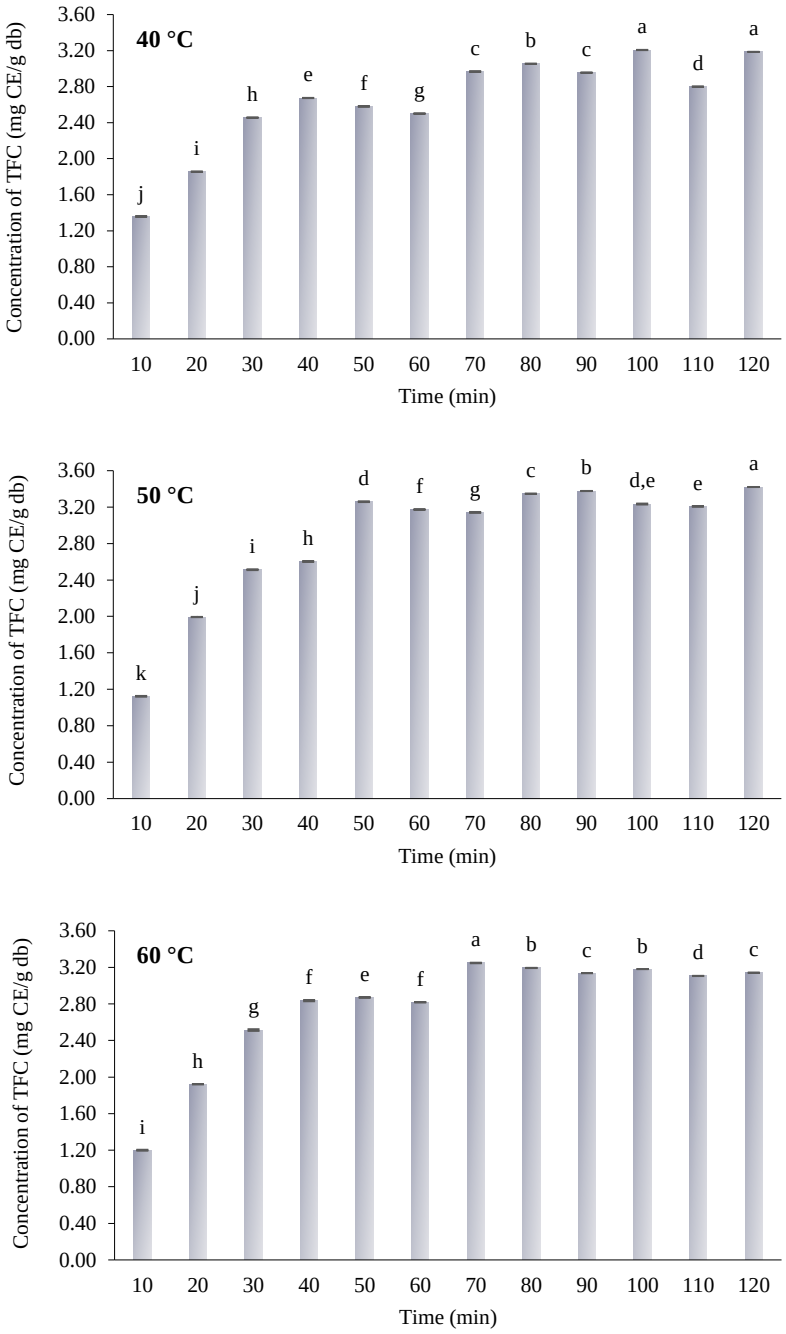

Figure 3 Concentration of TFC over time of *M. citrifolia* fruits at different temperatures. Values denote mean ± SD from three replicates. Different letters (within a bar) signify statistically significant distinctions (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

In this study, four mathematical models—the first-order kinetic, Peleg, power law, and two-site kinetic models—were assessed to elucidate the extraction behaviour of TFC in *M. citrifolia* fruits. Figure 4 presents a comparative analysis of the experimental and predicted extraction kinetic models of TFC at different temperatures. Table 2 simultaneously showcases the results aligning the experimental data with the extraction kinetic models for TFC in *M. citrifolia* fruits under different temperature conditions. The R^2 values showed variation as follows: 0.965 to 0.991 for the first-order kinetic model, 0.970 to 0.979 for the Peleg model, 0.926 to 0.949 for the power law model, and 0.971 to 0.991 for the two-site kinetic model, indicating strong correlation, with values close to 1 for all models. Additionally, R^2_{adj} values demonstrated fluctuation: 0.061 to 0.090 for the first-order kinetic model, 0.067 to 0.077 for the Peleg model, 0.019 to 0.044 for the power law model, and 0.068 to 0.090 for the two-site kinetic model. Furthermore, RMSE values spanned as follows: 0.412×10^{-2} to 1.437×10^{-2} for the first-order kinetic model, 0.932×10^{-2} to 1.282×10^{-2} for the Peleg model, 1.951×10^{-2} to 3.589×10^{-2} for the power law model, and 0.412×10^{-2} to 1.156×10^{-2} for the two-site kinetic model. The lowest χ^2 ranges were documented as follows: 0.004×10^{-3} to 0.049×10^{-3} for the first-order kinetic model, 0.000×10^{-3} to 0.052×10^{-3} for the Peleg model, 0.008×10^{-3} to 0.044×10^{-3} for the power law model, and 0.000×10^{-3} to 0.013×10^{-3} for the two-site kinetic model. MAE values also revealed ranges from 0.002 to 0.005 for the first-order kinetic model, 0.001 to 0.006 for the Peleg model, 0.002 to 0.006 for the power law model, and 0.000 to 0.003 for the two-site kinetic model.

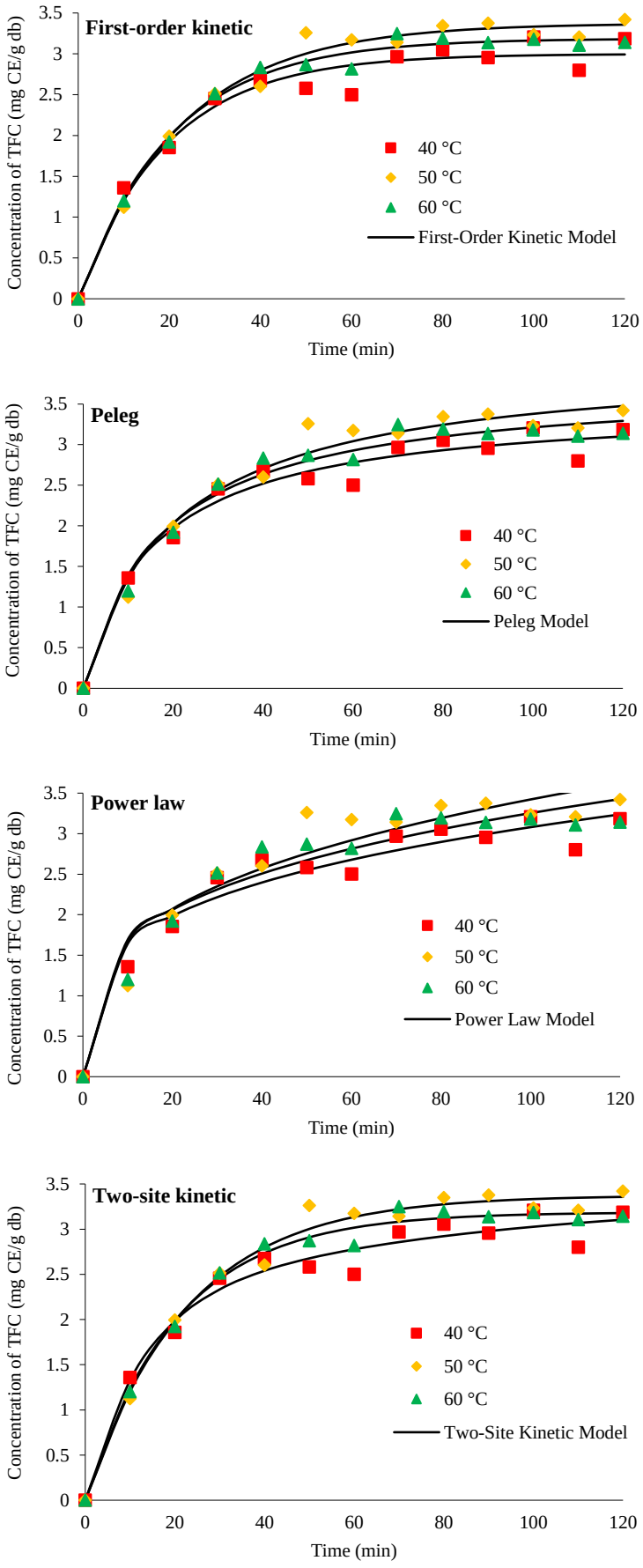

Figure 4 Comparison of experimental and predicted data for the extraction kinetic models of TFC in *M. citrifolia* fruits at different temperatures.

Table 2 Goodness of fit analysis for extraction kinetic models of TFC in *M. citrifolia* fruits at different temperatures.

Model	Temperature (°C)	Constant	R^2	R^2_{adj}	RMSE (10^{-2})	χ^2 (10^{-4})	MAE
First-order kinetic	40	C_e = 2.999 k = 0.051	0.965	0.061	1.437	0.049	0.005
	50	C_e = 3.374 k = 0.044	0.987	0.086	0.676	0.013	0.003
	60	C_e = 3.187 k = 0.049	0.991	0.090	0.412	0.004	0.002
Peleg	40	k_1 = 4.453 k_2 = 0.285	0.970	0.067	1.176	0.000	0.001
	50	k_1 = 4.916 k_2 = 0.247	0.975	0.072	1.282	0.052	0.006
	60	k_1 = 4.539 k_2 = 0.266	0.979	0.077	0.932	0.033	0.005
Power law	40	B = 0.873 n = 0.274	0.949	0.044	1.951	0.008	0.002
	50	B = 0.812 n = 0.312	0.926	0.019	3.589	0.044	0.006
	60	B = 0.879 n = 0.284	0.931	0.024	2.945	0.028	0.004
Two-site kinetic	40	C_w = 2.100 k_w = 0.082 C_d = 0.600 k_d = 0.013	0.971	0.068	1.156	0.000	0.000
	50	C_w = 2.506 k_w = 0.044 C_d = 0.346 k_d = 0.044	0.987	0.086	0.676	0.013	0.003
	60	C_w = 2.393 k_w = 0.049 C_d = 0.332 k_d = 0.049	0.991	0.090	0.412	0.004	0.002

Overall, the R^2 value obtained for the two-site kinetic model was highest at 60 °C (0.991), compared to 0.971 and 0.987 at 40 °C and 50 °C, respectively. A similar pattern is observed in the R^2_{adj} values of the model. Furthermore, the RMSE, χ^2, and MAE values for the two-site kinetic model were lower than those for the other models, although they were slightly higher at 40 °C. Consequently, the two-site kinetic model emerges as one of the most promising options for characterising the extraction kinetics of TFC in *M. citrifolia* fruits at temperatures of 40 °C, 50 °C, and 60 °C based on its superior goodness of fit. The concentrations of TFC in *M. citrifolia* fruits recovered at washing (C_w) and diffusion (C_d) stages, as described by the two-site kinetic model, varied with temperature. At 40 °C, C_w was 2.100 mg CE/g db, which increased to 2.506 mg CE/g db at 50 °C and slightly decreased to 2.393 mg CE/g db at 60 °C. Conversely, C_d decreased from 0.600 mg CE/g db at 40 °C to 0.346 mg CE/g db at 50 °C, and further to 0.332 mg CE/g db at 60 °C. These findings indicate that the extraction of TFC from *M. citrifolia* fruits is significantly influenced by temperature, resulting in varying concentrations recovered at different temperatures. Moreover, the extraction rate coefficients (k_w and k_d) for TFC extraction from *M. citrifolia* fruits also exhibited temperature-dependent behaviour. The coefficients for the extraction rate during the washing stage (k_w) decreased from 0.082 g db/mg CE·min at 40 °C to 0.044 g db/mg CE·min at 50 °C, and then slightly increased to 0.049 g db/mg CE·min at 60 °C. Similarly, the extraction rate coefficient during the diffusion stage (k_d) decreased from 0.013 g db/mg CE·min at 40 °C to 0.044 g db/mg CE·min at 50 °C, and remained constant at 0.049 g db/mg CE·min at 60 °C. These findings highlight a complex relationship between temperature and the extraction rate coefficients of TFC from *M. citrifolia* fruits, underscoring the need importance of precise temperature control in optimising TFC yield during the extraction process.

3.3 Effect of temperatures on antioxidant activity

The antioxidant activity of *M. citrifolia* fruits was assessed using the DPPH assay, as illustrated in Figure 5. A lower IC_{50} value indicates superior antioxidant properties. The results revealed a gradual decline in the IC_{50} value for DPPH inhibition, consistently decreasing as drying temperatures increased from 40 °C to 50 °C, with corresponding values ranging from 2626.75 ± 0.04 µg/mL to 1883.57 ± 0.02 µg/mL. Subsequently, *M. citrifolia* fruits dried at 60 °C exhibited the highest IC_{50} value for DPPH inhibition, registering at 3641.31 ± 0.05 µg/mL. In comparison, the positive control showed the lowest inhibition with an IC_{50} value of 95.11 ± 0.13 µg/mL. Notably, *M. citrifolia* fruits dried at 50 °C displayed a moderate potential for DPPH inhibition, indicating significant antioxidant activity.

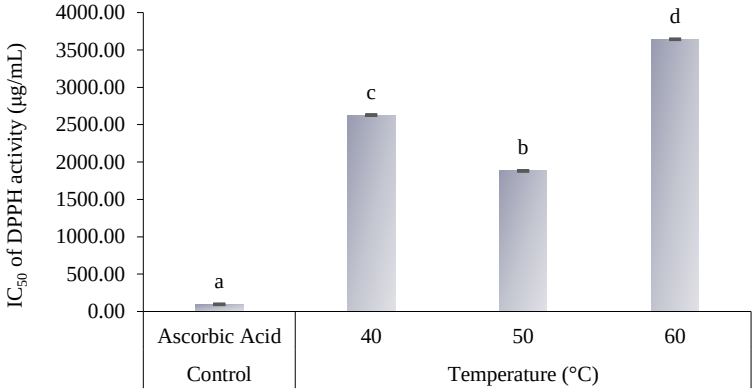

Figure 5 Antioxidant activity of *M. citrifolia* fruits assessed through IC_{50} values of DPPH inhibition at different temperatures. Values denote mean ± SD from three replicates. Different letters (within a bar) signify statistically significant distinctions (one-way ANOVA, Tukey's HSD test, $p < 0.05$). Ascorbic acid was denoted as a positive control.

4. Discussion

Different temperatures resulted in a range of maximum yields achieved at different times. The highest yield was obtained at 40 °C within 90 min, while at 50 °C and 60 °C, it was obtained within 110 min. These findings corroborate those of Rocha et al. [20], who optimised phenolic compounds from blueberry and jussara fruits, showing that peak concentrations of the compounds were reached between 45 to 90 min of extraction time. This suggests that extending extraction time may be necessary to enhance yields at higher temperatures (50 °C and 60 °C) [21]. Additionally, prolonged ultrasound exposure can lead to structural damage to the solute, thereby reducing extraction yield [22]. Moreover, the trend in TFC beyond the peak point at different temperatures is illustrated in Figure 2. The maximum TFC values were recorded at 40 ºC, 50 ºC, and 60 ºC after 120, 100, and 70 min, respectively. Kaleem et al. [23] studied Perlette grapes, revealing a quadratic relationship between extraction time and temperature on TFC, indicating the significant influence of both factors on TFC. Optimisation of the TFC from *Hypericum formosanum* showed that a 38-minute extraction time yielded the highest TFC [24]. These findings suggest that longer extraction times can enhance TFC. Therefore, based on these studies, increasing extraction time can potentially increase TFC. The observed decline in yield and TFC could impact the overall efficiency of the extraction process, affecting the modelling of yield in extraction kinetics. Nonetheless, this phenomenon does not directly reflect the actual kinetics but rather represents the rates of thermal degradation [25].

The model's goodness of fit can be assessed using a range of metrics. In the context of linear regression models, R^2 and R^2_{adj} are commonly employed as indicators of model performance [26]. R^2 indicates the proportion of variance in the dependent variable that can be attributed to the independent variables in the model. A higher R^2 value signifies a more accurate fit of the model to the data. R^2_{adj} is a modified version of R^2 that accounts for the number of predictors in the model. It penalises the inclusion of unnecessary predictors, providing a more precise measure of model fit when comparing models with varying numbers of predictors. Conversely, metrics like MAE, RMSE, and χ^2 are utilised to evaluate the precision of predictions or model efficacy [27, 28]. MAE gauges the average absolute difference between predicted and actual values, while RMSE quantifies the square root of the average squared deviation between predicted and actual values. Lower MAE and RMSE values signify superior predictive accuracy. χ^2 is a statistical test that contrasts observed and anticipated frequencies in different categories and is frequently applied in hypothesis testing. A lower χ^2 value denotes a superior alignment of the model with the observed data. To summarise, R^2 and R^2_{adj} are measures of model fit that evaluate the proportion of variance explained by the model, with higher values indicating a more precise fit. On the contrary, MAE and RMSE are gauges of prediction accuracy, with lower values signifying enhanced performance. χ^2 is a statistical test used for hypothesis testing, with lower values indicating a more accurate fit of the model to the observed data.

The two-site kinetic model emerges as a highly promising approach for characterising the extraction kinetics of yield and TFC in *M. citrifolia* fruits, owing to its exceptional goodness of fit at 40 °C, 50 °C, and 60 °C. The findings by Jamaludin et al. [29] align with the observed temperature-dependent trends in the concentration of TFC recovered during the washing and diffusion stages, as well as the extraction rate coefficients. Their study demonstrated the temperature-dependent extraction of alizarin, rutin, and scopoletin from *M. citrifolia* fruits using subcritical water extraction. The results indicated increased yields with higher temperatures, highlighting the influence of temperature on the extraction process. This parallels the observed trends in TFC concentration and extraction rate coefficients for *M. citrifolia* fruits, highlighting how temperature affects the kinetics of bioactive compound extraction. The temperature-dependent variations in TFC concentrations and extraction rate coefficients emphasise the importance of using kinetic models to understand the mechanisms of extracting bioactive compounds. This highlights the critical role of kinetic modelling in comprehending how temperature impacts the extraction process of *M. citrifolia* fruits. In addition, the two-site kinetic model has been widely employed to describe the kinetics of extracting active components like phenolic compounds from pistachio nuts [30] and glycyrrhizic acid in licorice [31]. Together, these studies demonstrate the applicability of the two-site kinetic model in describing the extraction kinetics of various bioactive compounds from medicinal plants. Therefore, applying the model can provide significant cost and time savings for manufacturers by optimising extraction processes and enhancing efficiency.

Antioxidants play a crucial role in reducing oxidative stress and preventing diseases. Their activity is often assessed by measuring the reduction of the DPPH radical, which causes a change in colour and decreased absorbance at 517 nm [32]. This method is commonly used to evaluate antioxidant activity in plant materials. The study highlights the potential of developing antioxidants from *M. citrifolia* fruits at different temperatures (Figure 5). Notably, extracted at 50 °C proved to be the most effective temperature for *M. citrifolia* fruits, as indicated by the lowest IC_{50} value for DPPH inhibition. Karle et al. [33] similarly found that the antioxidant activity of *Manilkara zapota* fruits was maximised using 70% ethanol extraction at 50 °C for 12 h. Likewise, optimal extraction from eggplant peel occurred at 45 °C for 45 min using a 70% solvent, resulting in DPPH inhibition values of 94.80%, compared to lower and higher

temperatures [34]. At 50 °C, the threshold temperature may prevent the degradation of thermolabile antioxidants, thus preserving higher activity levels. These findings suggest that 50 °C is optimal for achieving the highest antioxidant activity in *M. citrifolia* fruits. Flavonoids, a class of polyphenolic compounds renowned for their potent antioxidant properties, are known to neutralise reactive oxygen species and mitigate oxidative damage. The presence of flavonoids, phenolics, and polysaccharides in *M. citrifolia* contributes to its antioxidant properties [35]. Aryal et al. [36] also established a direct correlation between antioxidant activity in plant materials and the presence of flavonoids. In addition, flavonoids offer diverse health benefits, including anticancer and anti-inflammatory effects [37]. Higher temperatures enhance the efficiency of flavonoid extraction from fruits. Coupled with increased extraction efficiency and higher flavonoid concentrations at elevated temperatures, these nutritional attributes can enrich functional foods and nutraceutical products. Such products may provide additional health benefits, bolstering antioxidant status and potential safeguarding against chronic diseases [2]. Therefore, the elevation in flavonoid concentration due to higher temperatures has the potential to enhance the nutritional and therapeutic value of *M. citrifolia* fruits.

5. Conclusions

Overall, the highest yield of 1.43 ± 0.00 mg/mL was observed at 60 °C, while the maximum TFC of 3.43 ± 0.00 mg CE/g db was reached at 50 °C in *M. citrifolia* fruits. The two-site kinetic model effectively elucidated the extraction kinetics of yield and TFC, supported by their excellent goodness of fit. Moreover, the study revealed moderate antioxidant activity, with an impressive IC_{50} value of 1883.57 ± 0.02 µg/mL for DPPH inhibition at 50 °C. This highlights 50 °C as the optimal temperature for achieving the highest antioxidant activity. Collectively, these findings emphasise the potential of *M. citrifolia* fruits to advance standardised extraction kinetics, enhancing their therapeutic applications and offering cost and time savings for manufacturers.

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