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Microwave-Assisted Extraction's Kinetics of *Phycobiliprotein* from *Spirulina Platensis*: Influence of Citric Acid Concentration

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Abstract

Phycobiliproteins (PBP), such as *phycocyanin* (PC), *allophycocyanin* (APC), and *phycoerythrin* (PE), are photosynthetic pigments in *Spirulina platensis* with antioxidant potential for food and medicine. This study optimized microwave-assisted extraction (MAE) of PBP with citric acid (1, 2, and 3 %) as a green solvent to enhance yield and stability using kinetic approach. MAE increased solvent temperature from 30 to 60 °C, accelerating PBP release while preserving its bioactivity. Three kinetic models, Peleg, pseudo-first order (PFO), and pseudo-second order (PSO), were applied to analyze extraction kinetics. Peleg effectively described the initial phase, indicating that higher CA concentrations enhanced PBP release. PFO captured the diffusion-driven phase, while PSO provided the most accurate equilibrium prediction by considering adsorption and solvent-protein interactions. Among the models, PSO showed the highest extraction capacity ($Ce = 4.45 \times 10^{-2}$ mg/mL at 3 % CA), best R^2 (≥ 0.99), and lowest MAPE (3.45 %), confirming its superior fit at equilibrium. These findings highlight PSO as the best model for predicting maximum extraction, while Peleg and PFO remain relevant for the early and middle phases. The study confirms MAE with citric acid as an efficient, sustainable method for PBP extraction from *Spirulina platensis*.

Keywords: *Phycobiliprotein*, Citric acid, Peleg, Pseudo-first order, Pseudo-second order

Introduction

Blue-green algae (BGA) are microalgae of the cyanobacteria group, living organisms that have recently attracted much interest because of their use as food, cosmetic, and medicinal ingredients [1-4]. The superiority of microalgae bioactive compounds has encouraged their use in the food and health industries. Also, the rapid increase in population makes cyanobacteria such as blue-green algae a solution to be developed as a potential functional food need [5,6. *Spirulina platensis* is a type of cyanobacteria microalgae that is highly nutritious and beneficial due to its content of proteins, lipids, vitamins, and minerals [7-10]. The protein content in *Spirulina platensis* is very high compared to other types of microalgae, ranging from 60 - 70 % of its dry biomass. *Spirulina platensis* is also a source of bio pigments with high added value, such as *phycobiliproteins*, *chlorophyll,* and *carotenoids* [11-13].

Phycobiliproteins (PBP) are the main light-harvesting components found in cyanobacteria microalgae. The PBP are large, water-soluble supramolecular protein assemblies that play an essential role in light harvesting in Spirulina platensis and contribute to efficient photosynthesis. They can absorb light in a broad spectrum from 450 to 650 nm, making them versatile and efficient for capturing light energy for photosynthesis [14,15]. PBP not only acts as light

harvesting agents, but also act as bio pigments with various benefits. Based on their structure, characteristics, and light absorption quality, PBP are divided into 3 groups: *phycocyanin* (PC), *allophycocyanin* (APC), and phycoerythrin (PE) [16,17]. Most PBPs are *phycocyanins* with a dark blue pigment color, a type of protein with antioxidant, anti-inflammatory, and anticancer activity. Most PBPs are phycocyanins, characterized by a dark blue pigment, and possess antioxidant, anti-inflammatory, and anticancer properties. *Allophycocyanin* is a small component of *phycobiliprotein* biomolecules with a bright blue pigment color, and *phycoerythrin* has a bright pink pigment color [12,18,19].

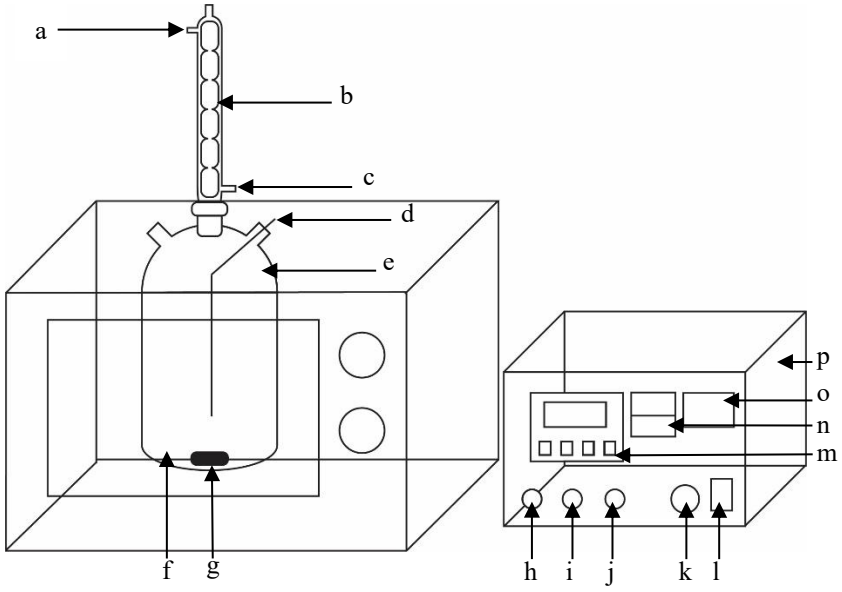
Extraction is an essential process supporting the collection and utilization of *phycobiliprotein* from *Spirulina platensis* as a source of potential antioxidant [20,21]. Various extraction techniques have been developed in many studies to extract bioactive compounds from microalgae biomass. MAE has shown significant potential in extracting bioactive compounds from *Spirulina platensis* which has benefits such as an efficient, fast process, and green process with reduced energy costs [22-25]. This method enhances the extraction of bioactive metabolites from Spirulina platensis. It allows for higher extraction yields due to efficient microwave penetration, which can disrupt cell walls and facilitate the release of bioactive compounds [26,27]. The success of the MAE method can be evaluated by kinetic approaches, which provide high accuracy in validation [28,29]. Kinetic modeling is crucial in optimizing microwave extraction processes across various applications [30,31].

Some parameters to evaluate the MAE process are temperature, solvent, and extraction time to maximize the extraction of bioactive compounds, including PBP [32]. This extraction method research was designed to develop technology for the renewable food industry by identifying, quantitatively analyzing, and evaluating target compounds [33]. These compounds have low stability capabilities, limiting their application in the industrial sector [34]. This biomolecule is very sensitive and has low stability to pH, heat, and light [35]. The application of solvents in the extraction process must maintain a high extract yield and be environmentally friendly [2,36]. Adopting green solvents, such as citric acid, in MAE processes reflects a broader trend towards sustainability in various industries, including biotechnology and agriculture [37,38]. Citric acid is one of the stabilizing agents that prevent the degradation of *phycobiliprotein* content [34,39]. Citric acid has been highlighted as a preferable food-grade additive for enhancing PBP extraction and stabilizing the compound, compared to other chemical preservatives, which may not be suitable for food applications due to toxicity concerns. The mechanism of preventing *phycocyanin* degradation by adding citric acid occurs because of the changes in physicochemical properties, which help reduce degradation caused by heat and acidity [35]. However, citric acid can also provide an acidic environment in solubilization that can lead to degradation of *phycobiliproteins.* The degradation pathway is modulated by environmental pH, with acidic conditions potentially leading to the degradation of phycobiliproteins to a certain extent [40,41]. Existing extraction methods for *phycobiliproteins* (PBP) often result in low efficiency and degradation, necessitating an improved technique. This study aims to optimize MAE with citric acid as a stabilizing agent, focusing on its role in enhancing extraction efficiency and PBP stability. The research evaluates the impact of citric acid concentration on heat generation, extraction kinetics, and yield, while comparing Peleg's model with other kinetic approaches to determine the best-fit model for describing PBP extraction dynamics.

Materials and methods

Materials in this research included dry biomass powder of *Spirulina platensis* Merck Spiruganik Polaris food which is 100 % organic *Spirulina platensis* from Jakarta, Indonesia. The reagents in this research were aquades and citric acid food grade merck Ensign from Ensign Industry, produced by Weifang Ensign Industry, Shandong Province, China. *A modified* Electrolux EMM2308X microwave oven with a 23L capacity from Indonesia, 800 W of microwave power, and 220 - 240 V/50 Hz voltage was used to perform MAE. The power of MAE, temperature, timer, magnetic stirrer's, and indicator lamp are all controlled by a control panel attached to the microwave. Up to 1600 RPM can be configured for the stirrer speed. The timer regulates the microwave radiation cycle. An additional temperature sensor is a type K thermocouple. During the extraction

process, the temperature is regulated by the CKC Tinner DH48S(H5CN) temperature controller and indicators, which are manufactured in China. The solvent-filled flask was placed inside the microwave chamber. A thermocouple was used to measure the solvent's temperature, which was adjusted with a knob. Utilizing aquadest as a solvent and citric acid as the extraction solvent, a magnetic stirrer was inserted into the bottom of the microwave flask to ensure the right amount of agitation. The extraction method with MAE is shown in **Figure 1**.



a: Water Outlet
b: Condensor
c: Water Inlet
d: Temperature Sensor
e: Connecting Tube
f: Extraction Flask
g: Magnetic Stirrer
h: Indicator Lamp 1
i: Indicator Lamp 2
j: Timer Button
k: Stirrer Button
l: On/Off Button
m: P & T Arranger
n: Timmer Controller
o: RPM Screen
p: Control Unit

Figure 1 The scheme of MAE process.

Extraction procedure

The extraction process was conducted with 10 g of dry biomass of *Spirulina platensis* in 300 mL of distilled water with various concentrations of citric acid 1, 2, and 3 % for 150 min in a flask in MAE at a temperature of 60 °C with a power percentage of 100 % and a capacity of 800 Watts. The content of the extracted *phycobiliprotein* was analyzed using a *spectrophotometer UV-Vis* with wavelengths of 562, 620, 652 nm to obtain the concentration of *phycocyanin*, *allophycocyanin,* and *phycoerythrin* levels. They were evaluated every 30 min with 3 repeated replications at each time point. The extracted sample was placed in a 15 mL centrifuge tube and then centrifuged at 4000 rpm for 10 min to separate the pellet and supernatant.

Analysis of *phycobiliprotein*

Phycobiliprotein of *Spirulina platensis* is classified into 3 types, namely *phycocyanin*, *allophycocyanin*, and *phycoerythrin*. Analysis of *phycobiliprotein* content in *Spirulina platensis* was measured using a N_2S visible spectrophotometer with an absorbance of 562, 620, and 652nm. The calculation formula for *phycocyanin* (PC), *allophycocyanin* (APC), and *phycoerythrin* (PE) content uses the following equation (Eqs. (1) - (3)) [12].

$$PC\left(\frac{mg}{mL}\right) = \frac{Abs\ 620\ nm - 0.474\ (Abs\ 652\ nm)}{5.34} \quad (1)$$

$$APC\left(\frac{mg}{mL}\right) = \frac{Abs\ 652\ nm - 0.208\ (Abs\ 620\ nm)}{5.09} \quad (2)$$

$$PE\left(\frac{mg}{mL}\right) = \frac{[Abs\ 562\ nm - 2.41\ (phycocyanin)] - 0.849\ (allophycocyanin)]}{9.62} \quad (3)$$

Analysis the profile of PBP content using several kinetic models

Peleg's model

To determine the rate of PBP extraction and the effect of citric acid concentration in preventing PBP degradation, temperature and microwave power need to be carried out using kinetic and modeling approaches to predict PBP content in the MAE process. One of the kinetic approaches used in the research is the Peleg model [42]. The Peleg model predicts the results and quality of *phycobiliprotein* extraction. The Peleg model in Eq. (4) is expected to provide good predictions of PC, APC, and PE content during MAE process. The value of C_t (mg/mL) is the PBP concentration at time *t* (min), C_0 is the initial PBP concentration (mg/mL) at time *t* = 0, t is the extraction time (min), K_1 is Peleg's model rate constant (mL.min/mg), and K_2 is Peleg's capacity constant (mL/mg). As the initial concentration of PBP in the solvent was 0, Eq. (4) is changed to Eq. (5).

$$C(t) = C_0 + \frac{t}{K_1 + K_2.t} \quad (4)$$

$$C(t) = \frac{t}{K_1 + K_2.t} \quad (5)$$

Peleg's rate constant K_1 and Peleg's capacity constant K_2 are correlated to the value of initial extraction rate (B_0) and equilibrium concentration (C_{eq}). The value of B_0 (6) and (7) was employed to determine the value of B_0 (mg/mL.min) and C_{eq} (mg/mL).

$$B_0 = \frac{1}{K_1} \quad (6)$$

$$C_{eq} = \frac{1}{K_2} \quad (7)$$

Pseudo-first order kinetic model

The *pseudo-first order* model is a kinetic model commonly used to describe changes in the concentration of a compound over time in the extraction process. This study applied this model to analyze the extraction of *phycobiliproteins* such PC, APC and PE from *Spirulina platensis* using MAE. This model assumes that the extraction rate is proportional to the difference between the compound concentration at the time *t* and the equilibrium concentration (C_e). This model is simpler to apply and faster for illustrating the extraction curve behaviour, as it involves only the extraction rate constant and initial concentration data. The *pseudo-first order* model provides an exponential relationship between the concentration of the extracted PBP at time *t* (C_t) and the equilibrium concentration (C_e), as described in Eqs. (8) - (9).

$$\frac{dC}{dt} = k_{pfo}(C_e - C_t) \quad (8)$$

To derive a more practical equation, we integrate Eq. (5) with the initial condition $C_t = C_0$ at $t = 0$, resulting in the following equation:

$$C_t = C_e(1 - e^{-k_{pfo}.t}) \quad (9)$$

Where (C_t) is the concentration of *phycobiliproteins* at the time (mg/mL), (C_e) is the equilibrium concentration (mg/mL), k_{pfo} is the extraction rate constant for the *pseudo-first order* kinetic model (1/min), and *t* is the extraction time (min). The *pseudo-first order* model is expected to provide a good prediction of the extraction dynamics and can be used to determine the efficiency of the MAE process in extracting PBP from *Spirulina platensis*, allowing for better control over the extraction process.

Pseudo-second order kinetic model

The *pseudo-second order* kinetic model is used to explain the change in the concentration of a substance over time during an extraction process, based on the assumption that the rate of extraction is proportional to the square of the difference between the substance's concentration at time *t* and its equilibrium concentration (C_e), as described in Eq (10) and (11). This model is applied to describe the extraction dynamics of *phycobiliproteins,* such as PC, APC and PE, from *Spirulina platensis* using MAE. Compared with the *pseudo-first order* and Peleg models, the *pseudo-second order* model provides a more complex relationship that accurately describes the extraction process. This model is better at describing the change in concentration over time, especially when the extraction rate is not linear initially and approaches equilibrium. The equation for this model is expressed as:

$$C_t = \frac{k_{pso}.t.C_e^2}{1 + k.t.C_e} \quad (10)$$

where (C_t) is the concentration of *phycobiliproteins* at time t (mg/mL), $(C_e)$is the equilibrium concentration (mg/mL), k_{pso} is the extraction rate constant for the *pseudo-second order* model (mg/mL.min), and t is the extraction time (min). To derive this equation, we begin with the general rate law for *pseudo-second order* reactions:

$$\frac{dC}{dt} = k_{pso}(C_t - C_e)^2 \quad (11)$$

We obtain the equation above by integrating this differential equation with the initial condition $C_t = C_0$ at $t = 0$. This model assumes that the extraction rate is proportional to the square of the concentration difference between the substance at time t and its equilibrium concentration (C_e). Since it is more complex than the *pseudo-first order model*, the *pseudo-second order* model better represents systems where the extraction rate significantly changes over time. This model predicts that the extraction rate is fast initially and gradually slows down as the system approaches equilibrium.

Evaluation the kinetic model

The comparison of the kinetic models was conducted using the sum of squared error (SSE) in Eq. (12), the root mean squared error (RMSE) in Eq. (13), the coefficient of determination (R^2) in Eq. (14), and the mean absolute percentage error (MAPE) in Eq. (15). The selected model was chosen based on the values of SSE, RMSE, R^2, and MAPE in Eqs. (12) - (15).

$$SSE = \sum_{i=1}^{n}(C_{t,i} - C_{p,i})^2 \quad (12)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(C_{t,i} - C_{p,i})^2} \quad (13)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(C_{t,i} - C_{p,i})^2}{\sum_{i=1}^{n}(C_{t,i} - mean\ (C_t))^2} \quad (14)$$

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\frac{|C_{t,i} - C_{p,i}|}{C_{t,i}} \quad (15)$$

where (C_t) is the observed concentration, (C_p) is the predicted concentration, $(C_{t.i})$ is the observed concentration at time t for the i-th data point, $(C_{p.i})$ is the predicted concentration at time t for the i-th data point, and n is the total number of data points.

Results and discussion

The temperature response in each concentration of solvent affected by microwave

The acceleration mechanism that occurs in microwave extraction is interesting to study. The solvent exposed to microwaves will experience heating caused by wave propagation, which results in collisions between particles. Heat generation through molecular vibrations in the solvent during extraction process occurs due to the interaction of solvent molecules with electromagnetic fields. The microwave energy is absorbed by the solvent and converted into heat energy. The amount of heat generated in the solvent varies depending on the concentration of citric acid in the solvent, as shown in **Figure 2** The higher the concentration, the faster the heat is generated. This phenomenon is because CA is a polar solvent that enhances the absorption of microwave energy. This rapid heating is crucial for the extraction process as it helps to break down cell walls and increase the diffusion of analytes into the solvent. The fast heat of microwaves causes a vibration that can destroy cell tissue [43].

Figure 2 illustrates the change in solvent temperature for 30 min in MAE at 800 W power and a maximum control temperature of 60 °C with each citric acid concentration of 1, 2, and 3 %. The higher concentration of citric acid showed the fastest temperature increase and reached 60 °C within 4 min. Meanwhile, the temperature increase at citric acid concentrations of 1 and 2 % tends to be slower to reach a temperature of 60 °C, the time required being 10 and 7 min, respectively. The concentration of citric acid affects the rate of heat generation in different concentration ranges by influencing the thermal properties of the solvent, the heat transfer mechanisms, and the impeller configurations used in the extraction process.

The phenomenon of increasing heat generation rate along with increasing citric acid concentration in the solvent can be attributed to several factors related to the physical and chemical properties of citric acid, such as

the dielectric properties of the solution. Increasing the concentration of citric acid results in increased dielectric properties that affect the heating rate. The dielectric constant is an important parameter that determines how effectively a solvent can absorb microwave energy and thus affects the heating rate during MAE [44].

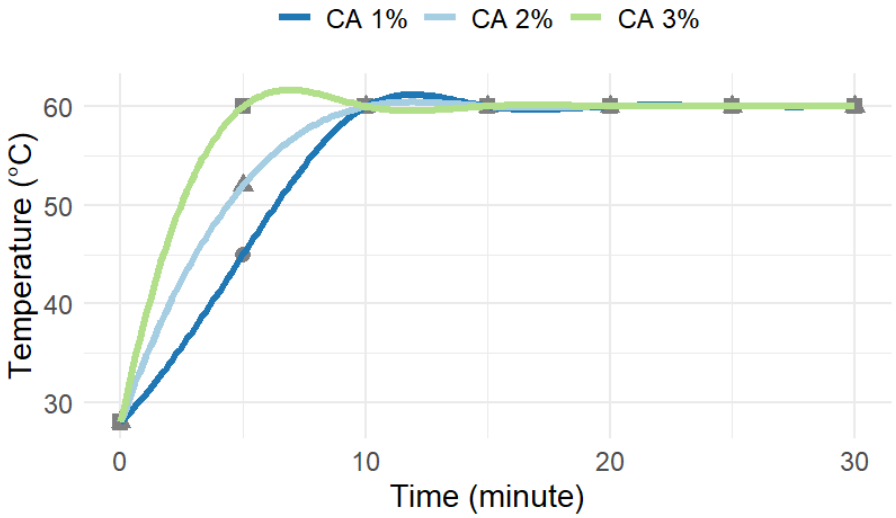


Figure 2 The temperature change on various citric acid concentrations in MAE.

The MAE mechanism uses microwave energy generated by a magnetron to heat a solvent in contact with the sample. A waveguide or beam component directs the microwaves into the solvent in the microwave chamber. Waveguides are designed to transmit microwave power efficiently by confining the electronic and magnetic fields and minimizing power loss due to radiation. The waveguide or beam is usually made of a conducting material designed to guide the microwave energy from the microwave source to the solvent in the chamber [42]. The microwave energy transfer into a solvent reflects the powers from the magnetron to the solvent. This energy transfer occurs through the interaction of microwave radiation with the solvent, which can be polar or non-polar. Polar solvents, such as water, methanol, and ethanol, absorb microwave radiation well and heat up rapidly due to the dipole rotation of their molecules. The rapid heating effect of MAE enhances solvent penetration into the sample matrix, facilitating the release of *phycobiliproteins*. This mechanism significantly shortens extraction time while preserving compound stability and yield [45,46].

The effect of citric acid concentration on the *phycobiliprotein* content during MAE

The enhancement of the MAE process with the addition of citric acid has been evaluated based on the increase in the concentration of 3 types of *phycobiliprotein* compounds, as seen in **Figure 3**. The *phycobiliprotein* (PBP) concentration increased rapidly during the initial extraction phase due to the rapid rise in temperature, enhancing solvent penetration. As the process continued, extraction slowed and eventually stabilized, indicating the system had reached equilibrium. This is associated with the increase in heat generation that occurs rapidly in the solvent so that it can accelerate the extraction process. The increase in heat generation will accelerate the solvent diffusion process into the material's pores to collect the target compound. This is because the increase in extraction temperature causes a decrease in the solvent's viscosity so that the solvent molecules' movement increases rapidly, and it is easier to enter the pores of the material and is easier to extract or dissolve *phycobiliprotein* compounds [47,48].

In addition to increasing the heat generation rate, citric acid has also been reported to help damage the cell pores in the material, thereby accelerating the diffusion

of solvents into the internal part of the material to dissolve *phycobiliprotein* compounds. Citric acid enhances cell membrane permeability by disrupting lipid-protein interactions, facilitating solvent penetration into intracellular compartments. Higher citric acid concentrations (3 %) resulted in greater membrane disintegration, leading to improved *phycobiliprotein* diffusion into the solvent. This is evidenced by the higher concentrations of PC, APC, and PE in 3 % citric acid compared to other concentrations [49].

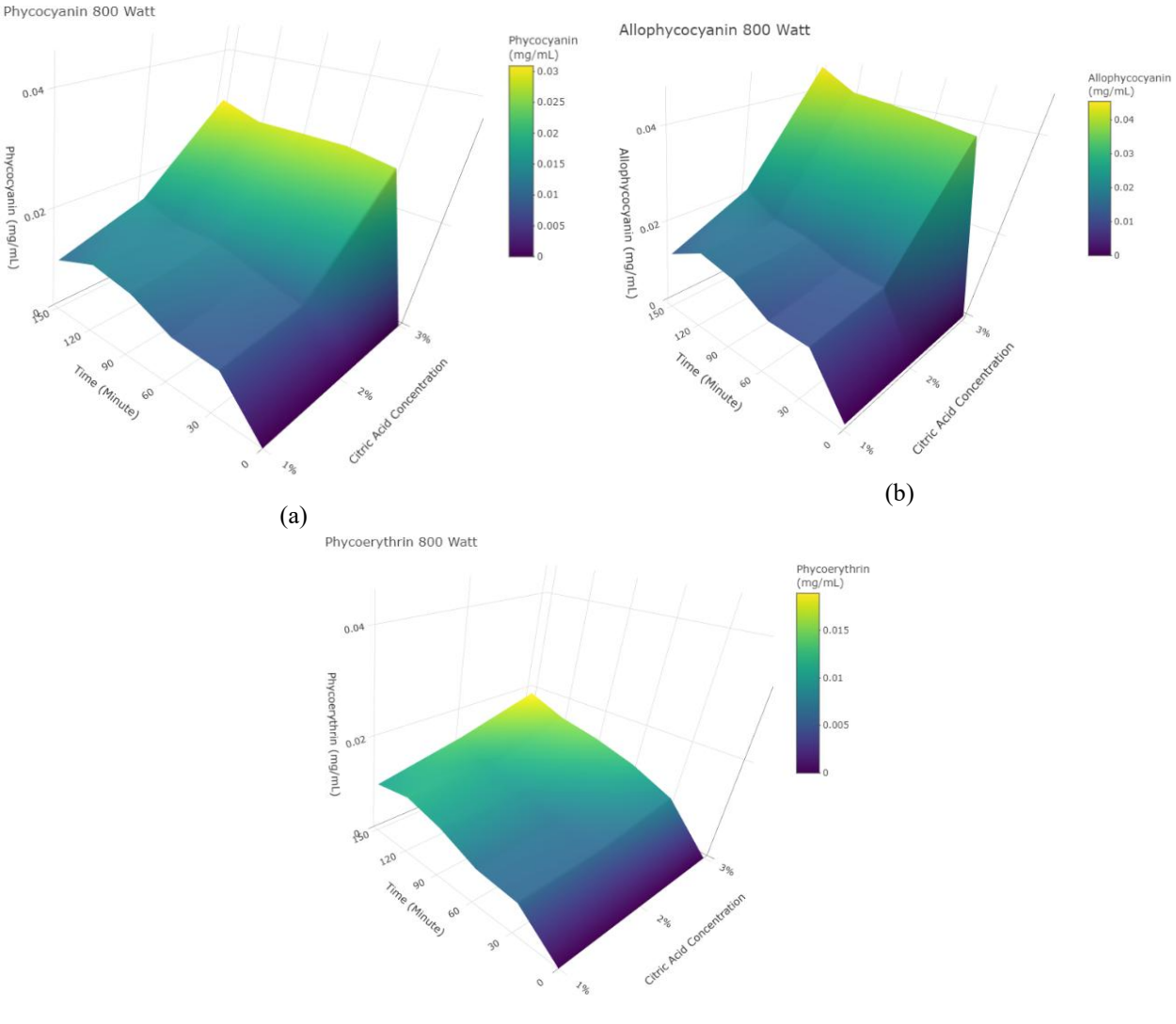


Figure 3 The PBP content in various CA concentrations of (a) *phycocyanin* (b) *allophycocyanin* (c) *phycoerythrin* in MAE.

Although disrupting cell structures can increase extraction efficiency, the same property can damage the structure of compounds if not carefully managed. However, Citric acid has also been reported as an effective stabilizing agent for *phycobiliproteins*, particularly *phycocyanin,* which is sensitive to environmental factors such as light, temperature, and pH. Research indicates that citric acid can significantly enhance the stability of *phycocyanin*, indicating its potential to prevent colour loss due to PBP degradation [35,50].

The PC, APC, and PE values from *Spirulina platensis* were tested for the main effect of extraction and interaction with citric acid concentration in preventing the degradation and loss of the content. The effect of CA concentration on preventing colour loss due

to PBP concentration is illustrated in **Figure 3**. Citric acid has been proven to prevent the degradation of content in all types of *phycobiliprotein* (PBP), namely *phycocyanin* (PC), *allophycocyanin* (APC), and *phycoerythrin* (PE). The concentration of CA 3 showed the most effective solvent condition to maintain colour loss from *phycocyanin* content. The highest PC concentration was obtained with the solvent condition of CA 3 % at 150 min with a value of 0.031 mg/mL. Likewise, in the solvent condition of CA 2 %, the highest PC concentration was obtained at 150 min with a value of 0.023 mg/mL. While in the solvent condition of CA 1 %, the highest PC concentration was obtained at 120 min with a value of 0.013 mg/mL. Solvent conditions at CA concentrations of 2 and 3 % showed more stable conditions in extracting and preventing degradation of PC content. While in CA 1 % conditions, the PC extraction trend tended to increase until min 120, then at min 150, the PC content decreased. This shows that the CA 1 % solvent condition is less effective in extracting and maintaining PC content in the MAE.

The solvent concentration of CA 3 % also showed the most effective conditions in extracting and preventing *allophycocyanin* (APC) and *phycoerythrin* (PE) content degradation. The APC content ranged from 0.038 to 0.045 mg/mL at a concentration of CA solvent 3 %, 0.015 to 0.021 mg/mL at a concentration of CA solvent 2 %, and 0.011 to 0.013 mg/mL at a concentration of CA solvent 1 %. The phenomenon of extraction kinetics at a concentration of CA 3% showed the most effective conditions for maintaining the loss of APC pigment compared to other solvent concentration conditions, which showed a trend of extraction that tended to fluctuate. Likewise, with the phenomenon of the extraction trend in *phycoerythrin* (PE), the concentration of CA 3 % showed the most stable and effective conditions in maintaining PE degradation compared to other concentrations with a fluctuating trend. PE content ranges from 0.009 to 0.019 mg/mL at 3 % CA concentration, 0.005 to 0.013 mg/mL at 2 % CA concentration, and 0.006 to 0.012 mg/mL at 1 % CA concentration.

The effect of citric acid concentration on pH Solvent

Citric acid plays an important role in lowering the pH of the extraction solution due to its acidic nature and ability to form complexes with various ions. The dissociation of citric acid in solution releases hydrogen ions (H^+), directly lowering the pH value. This property is often utilized in extraction to create optimal conditions for specific chemical reactions or separations [51,52].

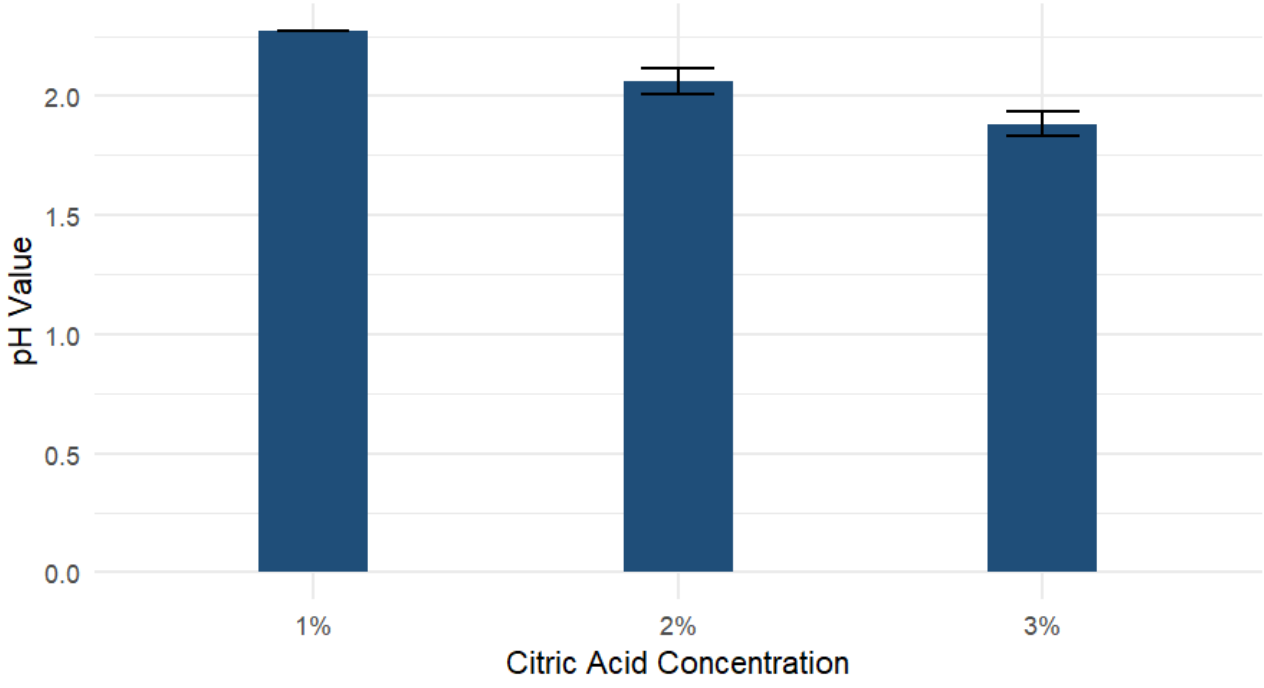


Figure 4 The pH Value of solvent on various citric acid concentrations in MAE.

Based on the data, increasing the concentration of citric acid causes a significant decrease in the solutions's pH value. At a concentration of 1 %, the pH value obtained was 2.27, indicating that the solution was in a stable condition with relatively moderate acidity. When the concentration increased to 2 %, the pH value dropped to 2.06, indicating an increase in the solution's acidity. This decrease became more significant at a concentration of 3 %, where the pH value reached 1.88.

Extreme pH conditions, such as at a CA concentration of 3 %, are one of the challenges in the extraction process of *phycobiliproteins* (PC, APC, and PE). This is due to the nature of PC, APC, and PE, which are low-stable to extreme pH and temperature [53]. In addition, the extraction method used, namely MAE, is a type of heat-based extraction that has the potential to cause degradation of PC, APC, and PE. Although high citric acid concentrations (3 %) significantly lower pH (1.88), their chelating and antioxidant properties outweigh the negative effects of extreme acidity by stabilizing *phycobiliproteins*. The ability of citric acid to bind metal ions reduces oxidative stress, while its pH-buffering capacity prevents excessive protein denaturation during microwave heating [54].

As previously explained, citric acid plays an essential role in increasing the effectiveness of *phycobiliprotein* extraction. The effectiveness of MAE on PBP occurs through the synergistic mechanism of citric acid, which accelerates the extraction process while preventing PBP degradation. Citric acid increases the extraction rate at higher concentrations by strengthening microwave heating. This is because citric acid is a weak acid that allows more excellent absorption of microwave energy. The interaction of citric acid ions with the microwave field causes molecular vibrations, essential for generating heat during microwave-assisted processes. The absorbed microwave energy is converted into kinetic energy, increasing molecular vibrations and the system's heat energy. This increase in energy produces faster and more even heat, thus accelerating cell wall disruption in microalgae [55,56].

Along with accelerating extraction, citric acid acts as an effective PBP stabilizing agent. In extreme conditions such as high heating, proteins are susceptible to degradation, including denaturation and oxidation. Citric acid acts as a chelating agent that helps bind metal ions that can trigger oxidation reactions and cause PBP degradation. By reducing the availability of metal ions, citric acid can protect the protein structure from oxidative reactions during the extraction process, maintaining the integrity of *phycobiliproteins* [57-59]. Thus, using citric acid provides 2 main advantages: accelerating the extraction through a more efficient heating mechanism and preventing *phycobiliprotein* degradation through a chemical stabilization mechanism [60,61].

When different treatments and extraction solvents were tested, the extraction yield of bioactive compounds varied widely. PBP extraction yields can vary depending on the method and condition used [62,63]. These results evaluate the most efficient extraction conditions with citric acid solvent for *phycobiliprotein* content of *Spirulina platensis* with high extraction yield.

As previously known, a concentration of 3 % CA solvent effectively extracted and prevented degradation of PBP content during the MAE process at extraction conditions of 800 W power and 60 °C. In addition to the rapid heat effect caused by adding CA to the solvent, CA can maintain PBP content in the MAE process to prevent degradation caused by the heat generated [64].

Extraction kinetics of PC, APC and PE during MAE

Kinetic approaches are vital in optimizing PBP extraction in cyanobacteria such as *Spirulina platensis* [65,66]. By using a kinetic modelling approach, this study was able to predict the increase in PBP accumulation by understanding the impact of factors such as CA concentration on the mechanism of PBP extraction by microwave. Kinetic modelling also helps to overcome the challenge of low extract productivity and allows for higher prediction of extract yield through a robust optimization approach [64,67]. In extraction kinetics studies, more than 1 model is often applied to understand the extraction mechanism better. No single kinetic model can explain the entire complexity of the extraction process. Using various kinetic models lets us obtain a more comprehensive picture of the extraction rate and the underlying mechanisms. **Figure 5**. Shows the performance of 3 different kinetic models, namely Peleg's model, *pseudo-first order* model, and *pseudo-second order* model, in explaining the extraction

kinetics of *phycobiliproteins* (PBP), including *phycocyanin* (PC), *allophycocyanin* (APC), and *phycoerythrin* (PE) from *Spirulina platensis* using MAE at various concentrations of citric acid (CA). The results presented in **Tables 1** - **3**. provide model parameters for each model, including extraction rate constant and maximum extraction capacity with SSE, RMSE, R^2, and MAPE parameters as model evaluation materials.

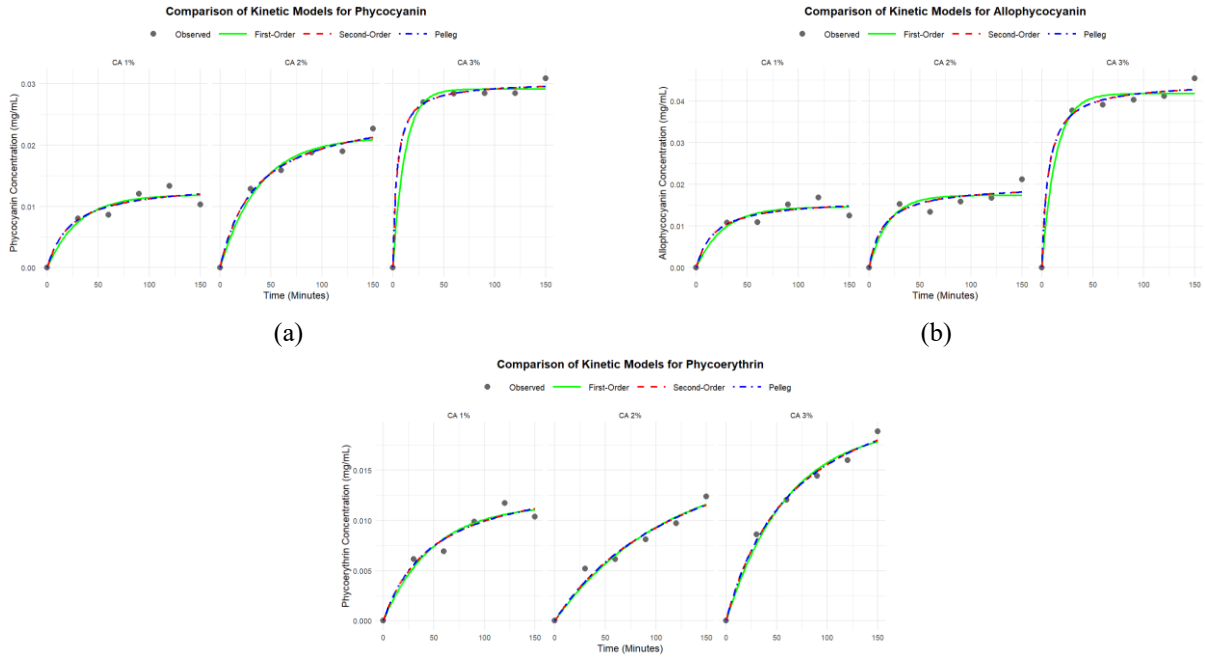


Figure 5 The observed and predicted of PBP content in various CA concentration of (a) *phycocyanin* (b) *allophycocyanin* (c) *phycoerythrin*.

The extraction process, as simulated by the Peleg model, begins with solvent molecules penetrating the solid matrix, leading to the dissolution of extract particles into the solvent. This empirical kinetic Peleg model effectively captures the extraction mechanism, transitioning from the initial fast extraction phase to the slower phase as equilibrium approaches. This model uses parameters such as the initial rate of extraction (B_0) and the maximum extraction capacity (C_e) to predict the yield and quality of *phycobiliproteins* [68-70]. As seen in **Table 1**, the initial extraction rate of the Peleg model (B_0) increases in line with the rise in the concentration of citric acid in the extraction solution. This shows that citric acid has been proven to stabilize the content of PC, APC, and PE by interacting with their molecular structure. In particular, anio citrate disrupts the water structure around PC, APC, and PE, which helps maintain their stability during the extraction process. This interaction is critical to maintaining the functional properties of pigments in the extraction process with high temperatures and extreme pH conditions [70-72]. The parameters of the Peleg model describe the extraction rate and the prevention of thermal and extreme pH degradation. From the effect of Citric Acid concentration on *phycocyanin* (PC) content, the highest B_0 value is 7.63×10^{-4} mg/mL. min at a CA concentration of 3 %, and the lowest value is 5.95×10^{-4} mg/mL. min at a CA concentration of 1 %. In the effect of CA concentration on *allophycocyanin* (APC) content, the highest B_0 value, is 1.39×10^{-3} mg/mL. min at a CA concentration of 2 %, and the lowest value is 7.14×10^{-4} mg/mL. min at a CA concentration of 3 %. Meanwhile, the effect of CA concentration on *Phycoerythrin* (PE) content, the highest B_0 value is 3.85×10^{-4} mg/mL. min at 3 % CA concentration and the lowest value is 1.57×10^{-4} mg/mL. min at 2 % CA concentration. The highest B_0 value indicates the optimal conditions for obtaining the highest PC, APC, and PE extraction content using a citric acid solution. The maximum capacity value (C_e) of Citric acid at the highest PC content is 3.03×10^{-3}

mg/mL at 3 % CA concentration and the lowest value (C_e) is 1.39 × 10^{-4} at 1 % CA concentration. The maximum capacity value (C_e) of Citric acid at the highest APC content is 4.45 × 10^{-3} mg/mL at 3 % CA concentration and the lowest value (C_e) is 1.65 × 10^{-4} mg/mL at 1 % CA concentration. Also the maximum capacity value (C_e) of CA at the highest PE content is 2.62 × 10^{-4} mg/mL at 3 % CA concentration and the lowest value (C_e) is 1.49 × 10^{-4} mg/mL at 1 % CA concentration. The highest value (C_e) indicates the maximum extraction capacity of PC, APC, and PE from *Spirulina platensis* using MAE.

Table 1 Peleg's model parameter for extraction kinetics in various CA concentration.

CA	PBP	K_1 (mL. min/mg)	K_2 (mL/mg)	B_0 (mg/mL. min)	C_e (mg/mL)	SSE	RMSE	R^2	MAPE %
1 %	PC	1.68 × 10^3	72.20	5.95 × 10^{-4}	1.39 × 10^{-4}	8.67 × 10^{-6}	1.20 × 10^{-3}	0.922	11.22
	APC	1.05 × 10^3	60.69	9.52 × 10^{-4}	1.65 × 10^{-4}	1.67 × 10^{-5}	1.67 × 10^{-3}	0.904	4.75
	PE	3.35 × 10^3	67.17	2.99 × 10^{-4}	1.49 × 10^{-4}	3.98 × 10^{-6}	8.14 × 10^{-4}	0.956	2.08
2 %	PC	1.33 × 10^3	38.15	7.52 × 10^{-4}	2.62 × 10^{-4}	4.73 × 10^{-6}	8.88 × 10^{-4}	0.985	12.57
	APC	7.21 × 10^2	50.42	1.39 × 10^{-3}	1.98 × 10^{-4}	2.25 × 10^{-5}	1.93 × 10^{-3}	0.914	12.04
	PE	6.38 × 10^3	44.03	1.57 × 10^{-4}	2.27 × 10^{-4}	3.34 × 10^{-6}	7.46 × 10^{-4}	0.963	3.45
3 %	PC	1.31 × 10^2	32.97	7.63 × 10^{-4}	3.03 × 10^{-3}	2.87 × 10^{-6}	6.92 × 10^{-4}	0.996	9.50
	APC	1.40 × 10^2	22.49	7.14 × 10^{-4}	4.45 × 10^{-3}	1.23 × 10^{-5}	1.43 × 10^{-3}	0.991	10.69
	PE	2.60 × 10^3	38.21	3.85 × 10^{-4}	2.62 × 10^{-4}	1.83 × 10^{-6}	5.53 × 10^{-4}	0.992	4.13

*Abbreviations: K_1: Peleg's model rat contant; K_2: Peleg's capacity contant; B_0: Initial extraction rate; *Ce*: Equilibrium concentration; SSE: Sum of Squared Errors; RMSE: Root Mean Squared Errors; R^2: Coefficient of determination; MAPE: Mean Absolute Percentage Error.

The *pseudo-first order* kinetic model is used here to describe the mass transfer in the extraction process, where the extraction rate is determined by the diffusion of PBP from the cellular matrix to the solvent [73,74]. This model states that the rate of PBP extraction is directly proportional to the difference between the maximum capacity (C_e) and the amount of PBP extracted at a given time. As time passes, the extraction rate decreases as less PBP compound remains in the cellular matrix to be extracted, eventually reaching equilibrium.

The results presented in **Table 2**. showed that at a concentration of 1 % Citric Acid (CA), the maximum extraction capacity (C_e) for PC was 1.16 × 10^{-2} mg/mL, with an extraction rate constant (k_{pfo}) of 2.05 × 10^{-2} 1/min. Increasing the concentration of Citric Acid to 2 % increased the C_e to 1.29 × 10^{-2} mg/mL, and the extraction rate constant (k_{pfo}) increased to 2.38 × 10^{-2} 1/min. At 3% concentration, C_e reached 1.57 × 10^{-2} mg/mL but k_{pfo} decreased slightly to 1.77 × 10^{-2} 1/min. The decrease in the extraction rate constant at this high concentration indicates that although the extraction capacity increased, the extraction rate began to slow down due to solvent saturation. *Allophycocyanin,* with slightly different characteristics, showed that at 1 % CA, C_e was 1.56 × 10^{-2} mg/mL, with k_{pfo} of 9.03 × 10^{-3} 1/min. When the Citric Acid concentration increased to 2 %, C_e increased to 1.81 × 10^{-2} mg/mL, but k_{pfo} decreased slightly to 7.23 × 10^{-3} 1/min. At 3% CA, C_e reached 2.23 × 10^{-2} mg/mL, but k_{pfo} further decreased to 4.75 × 10^{-3} 1/min. Although the maximum extraction capacity increased, the extraction rate decreased at higher concentrations, possibly due to the influence of increasingly inhibited diffusion or solvent-matrix interactions. As for PE, at 1 % CA, C_e was 1.95 × 10^{-2} mg/mL, and k_{pfo} was 1.66 × 10^{-2} 1/min. At 2 % CA, C_e increased to 2.24 × 10^{-2} mg/mL, and k_{pfo} was 1.49 × 10^{-2} 1/min. At 3 % CA concentration, C_e increased to 2.58 × 10^{-2} mg/mL, with k_{pfo} increasing to 2.40 × 10^{-2} 1/min, indicating that the PE extraction rate continued to increase with increasing citric acid concentration. This suggests that PE is more responsive to increasing solvent concentration, allowing for increased extraction efficiency at higher concentrations.

The pseudo-first-order model effectively describes the initial diffusion-dominated extraction phase.

However, it has limitations in capturing the full complexity of the extraction mechanism, particularly during the equilibrium phase. This model assumes that diffusion is the only factor controlling the extraction rate [75,76]. In contrast, the extraction process is also influenced by the chemical interaction between the solvent (citric acid) and *phycobiliprotein* molecules and the physical conditions of the *Spirulina platensis* matrix. Therefore, although the *pseudo-first order* model provides a good description of the initial extraction rate, it cannot fully explain the overall extraction dynamics. After applying the *pseudo-first order* kinetic model, we can proceed with a theoretical approach to further understand the extraction mechanism.

Table 2 *Pseudo-First order* kinetic model parameter for extraction kinetics in various CA concentration.

CA	PBP	C_e (mg/mL)	k_{pfo} (1/min)	SSE	RMSE	R^2	MAPE
1 %	PC	1.16×10^{-2}	2.05×10^{-2}	4.08×10^{-6}	8.25×10^{-4}	0.955	9.87
	APC	1.56×10^{-2}	9.03×10^{-3}	3.75×10^{-6}	7.90×10^{-4}	0.959	11.08
	PE	1.95×10^{-2}	1.66×10^{-2}	3.09×10^{-6}	7.18×10^{-4}	0.986	5.58
2 %	PC	1.29×10^{-2}	2.38×10^{-2}	3.33×10^{-6}	9.13×10^{-4}	0.964	11.74
	APC	1.81×10^{-2}	7.23×10^{-3}	3.50×10^{-6}	6.72×10^{-4}	0.965	8.54
	PE	2.24×10^{-2}	1.49×10^{-2}	2.72×10^{-6}	6.18×10^{-4}	0.989	3.25
3 %	PC	1.57×10^{-2}	1.77×10^{-2}	2.96×10^{-6}	7.26×10^{-4}	0.983	8.92
	APC	2.23×10^{-2}	4.75×10^{-3}	2.17×10^{-6}	5.37×10^{-4}	0.973	5.03
	PE	2.58×10^{-2}	2.40×10^{-2}	2.04×10^{-6}	4.51×10^{-4}	0.994	1.98

*Abbreviations: C_e: Equilibrium concentration; k_{pfo}: Pseudo-first order rate constant; SSE: Sum of Squared Errors; RMSE: Root Mean Squared Errors; R^2: Coefficient of determination; MAPE: Mean Absolute Percentage Error.

In theory, diffusion is still considered the main factor in the extraction process. However, other factors, such as temperature and the properties of *phycobiliprotein* molecules, such as size and polarity, also need to be considered. In MAE-based extraction, microwaves selectively heat the solvent, accelerating the diffusion of *phycobiliproteins* from inside the cell to the solvent [27]. In addition, citric acid stabilizes the molecular structure of *phycobiliproteins*, preventing thermal degradation or degradation due to extreme pH and allowing for more efficient extraction. As a next step, the *pseudo-second order* kinetic theory can be used to describe more deeply the chemical interactions between solvents and *phycobiliproteins*. This model is more suitable for describing conditions where the extraction rate depends on diffusion and adsorption or chemical bonds between the solvent and *phycobiliprotein* molecules [77,78]. We can obtain a more comprehensive picture of the extraction mechanism using various kinetic models such as *pseudo-first order* and *pseudo-second order*. We can optimize extraction conditions to increase extract yields.

The *pseudo-second order* kinetic model provides a more comprehensive understanding of the *phycobiliprotein* (PBP) extraction mechanism by considering both diffusion and adsorption or chemical interactions between *phycobiliproteins* and the solvent (citric acid) [79]. This model assumes that the extraction rate is influenced by the available PBP concentration and the solvent's ability to interact with the PBP molecules. Unlike the *pseudo-first order* model which primarily describes the diffusion dominated phase, the *pseudo-second order* model is more effective in explaining the equilibrium phase where the interactions between the solvent and PBP molecules control the extraction rate [80]. The application of the *pseudo-second order* kinetic model to the extraction data, as presented in **Table 3**. reveals significant insights into the mechanism of PBP extraction at different citric acid concentrations.

For PC, at 1 % citric acid, the maximum extraction capacity (C_e) is 1.39×10^{-2} mg/mL, with a rate constant (k_{pso}) of 3.112 1/min. Increasing the citric acid concentration to 2 % results in a higher C_e 2.62×10^{-2}

mg/mL but a lower k_{pso} value 1.091 1/min, suggesting that although more PC can be extracted, the rate of extraction slows due to possible interactions between PC molecules and the solvent. At 3 % citric acid, C_e further increases to 3.03 × 10⁻² mg/mL. k_{pso} reaches its highest value 8.324 1/min, indicating a more favourable interaction between PC and the solvent, leading to a more efficient extraction process. For APC, the results follow a slightly different trend. At 1 % citric acid, C_e is 1.65 × 10⁻² mg/mL, with a k_{pso} value of 3.493 1/min. Increasing the citric acid concentration to 2 % increases C_e to 1.98 × 10⁻² mg/mL, with a slightly higher $k_{pso}$of 3.528 1/min. However, at 3 % citric acid, C_e significantly increases to 4.45 × 10⁻² mg/mL, but k_{pso} remains relatively stable at 3.610 1/min. This suggests that while the solvent can extract more APC, the adsorption or stabilization of APC within the solvent medium becomes a more dominant factor in controlling the extraction rate. The extraction behaviour of PE under the *pseudo-second order* model exhibits a unique pattern. At 1 % citric acid, C_e is 1.50 × 10⁻² mg/mL, with a k_{pso} value of 1.348 1/min. At 2 % citric acid, C_e increases to 2.27 × 10⁻² mg/mL, but k_{pso} drops significantly to 0.304 1/min, indicating a slower extraction rate as equilibrium approaches. At 3% citric acid, C_e reaches 2.62 × 10⁻² mg/mL. However, k_{pso} remains relatively low at 0.560 1/min, suggesting that PE extraction is less dependent on rapid diffusion and more influenced by solvent interactions and stabilization effects.

Table 3 *Pseudo-second order* kinetic model parameter for extraction kinetics in various CA concentration.

CA	PBP	C_e (mg/mL)	k_{pso} (mg/mL.min)	SSE	RMSE	R^2	MAPE
1 %	PC	1.39 × 10⁻²	3.112	8.68 × 10⁻⁶	1.20 × 10⁻³	0.922	11.22
	APC	1.65 × 10⁻²	3.493	1.67 × 10⁻⁵	1.67 × 10⁻³	0.904	12.57
	PE	1.50 × 10⁻²	1.348	3.98 × 10⁻⁶	8.15 × 10⁻⁴	0.956	9.50
2 %	PC	2.62 × 10⁻²	1.091	4.73 × 10⁻⁶	8.88 × 10⁻⁴	0.985	4,75
	APC	1.98 × 10⁻²	3.528	2.25 × 10⁻⁵	1.94 × 10⁻⁴	0.914	12.04
	PE	2.27 × 10⁻²	0.304	3.34 × 10⁻⁶	5.53 × 10⁻⁴	0.963	10.69
3 %	PC	3.03 × 10⁻²	8.324	2.87 × 10⁻⁶	6.92 × 10⁻⁴	0.996	2.08
	APC	4.45 × 10⁻²	3.610	1.24 × 10⁻⁶	1.44 × 10⁻⁴	0.991	3.45
	PE	2.62 × 10⁻²	0.560	1.83 × 10⁻⁶	5.53 × 10⁻⁴	0.992	4.13

*Abbreviations: C_e: Equilibrium concentration; k_{pso}: Pseudo-second order rate contant; SSE: Sum of Squared Errors; RMSE: Root Mean Squared Errors; R^2: Coefficient of determination; MAPE: Mean Absolute Percentage Error.

After applying the *pseudo-second order* kinetic model, further understanding of the extraction mechanism can be gained by considering other factors such as solvent polarity, pH stability, and structural changes in PBPs during extraction. The interaction between citric acid and PBP molecules may involve a complexation effect. This study used 3 kinetic models, namely Peleg, *pseudo-first order*, and *pseudo-second order*, to understand the *phycobiliprotein* (PBP) extraction mechanism from *Spirulina platensis*. The Peleg model effectively describes the early extraction phase, indicating that increasing citric acid concentration accelerates the initial release of PBP into the solvent. However, this model does not consider the diffusion mechanism or chemical interactions, making it less able to explain the equilibrium phase [81,82]. The *pseudo-first order* model is more suitable for explaining the early to middle phase of extraction, where diffusion is the main factor in determining the extraction rate [83]. However, this model does not capture the interaction between the solvent and PBP, which becomes more dominant when the extraction reaches equilibrium. Meanwhile, the *pseudo-second order* model provides a more comprehensive description because it considers

the adsorption and chemical interactions between the solvent and PBP [84]. This model showed a higher maximum extraction capacity (C_e) compared to the other 2 models, especially at a citric acid concentration of 3 %, and had the highest R^2 value (≥ 0.99), indicating the best fit with the experimental data. Thus, although the Peleg and *pseudo-first order* models are better at describing the initial phase of extraction, the *pseudo-second order* is more accurate in describing the equilibrium phase, making it a more appropriate model for predicting the maximum extraction capacity of PBP in MAE systems and mobilising the extracted protein, thereby preventing degradation and increasing the extraction yield. In addition, **Figure 6** shows how the representation of the accuracy of the prediction data generated by each kinetic equation model with the obtained observation data.

Based on the analysis of kinetic parameters, the *pseudo-second order* model was shown to have the best performance in describing the extraction of *phycobiliproteins* from *Spirulina platensis*, with the highest R^2 value (≥ 0.99) and the lowest MAPE (4.13 % at CA 3 %), indicating a perfect match with the experimental data, especially at the equilibrium phase. The Peleg model was quite effective in explaining the early phase of extraction, especially at CA 3 %, where the R^2 value reached 0.996, but at lower CA concentrations, its accuracy decreased. Meanwhile, the *pseudo-first order* model was better at explaining the early to the middle phase of extraction, with a reasonably good R^2 value, but less accurate in predicting the equilibrium phase than the *pseudo-second order* model. Therefore, the pseudo-second order model is the best choice if the analysis aims to understand the extraction mechanism thoroughly, especially when considering the solvent-protein interaction. At the same time, the Peleg and *pseudo-first order* models are still relevant to understanding the early phase and the role of diffusion in the extraction process.

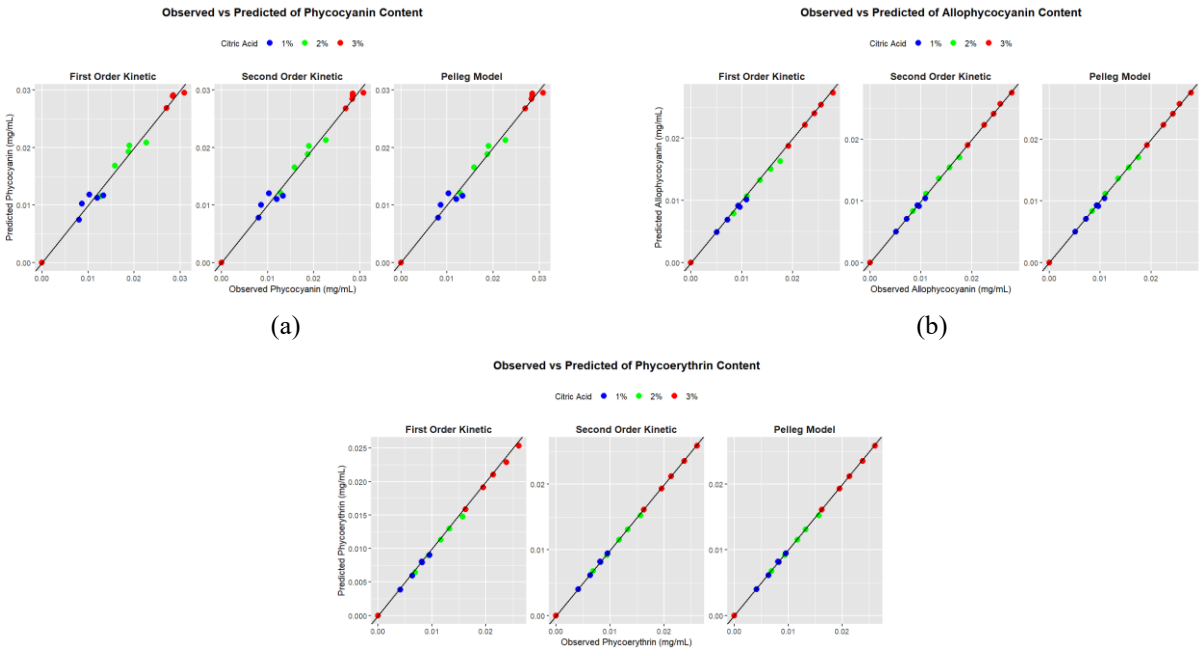


Figure 6 Correlation of the observed and predicted PBP content in various CA concentration of (a) *phycocyanin* (b) *allophycocyanin* (c) *phycoerythrin.*

Conclusions

The CA concentration affected the temperature increase and PBP extraction from *Spirulina platensis* using MAE. Higher CA concentrations in MAE enhanced heat generation, accelerating extraction by disrupting cell structures, increasing solvent penetration, and stabilizing PBPs in the solvent. The concentration of CA also affects the concentration and yield of each pigment of PC, APC, and PE. This study confirms that citric acid effectively prevents PBP thermal degradation in MAE, providing a quantified approach to optimizing solvent conditions for enhanced extraction yield. Additionally, increasing CA concentration decreased the pH value of the solution, yet this did not lead to a significant degradation of PBP, which remained stable throughout the extraction process. The *pseudo-second order* model is the most accurate in describing *phycobiliprotein* extraction using MAE, with the lowest SSE and RMSE and the highest R^2 ($\geq$ 0.99), indicating the best fit with experimental data. Additionally, its lower MAPE, especially at 3 % CA, confirms its superiority in extraction prediction. While the Peleg and *pseudo-first order* models remain relevant for understanding the initial phase and diffusion mechanisms**,** *pseudo-second order* excels in describing the equilibrium phase, making it the best model for optimizing *phycobiliprotein* extraction.

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