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Batch extraction of β-carotene from Gac (*Momordica cochinchinensis* Spreng) fruit peel

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Abstract

β-carotene, a prominent provitamin A carotenoid, functions both as an essential antioxidant beneficial to human health and as a natural food colorant. Gac (*Momordica cochinchinensis* Spreng) fruit peel, often discarded as biomass waste, represents a promising source of β-carotene. Extracting β-carotene from Gac peel can significantly enhance the value of this waste material. In this study, a conventional solid-liquid method was employed to extract β-carotene from Gac peel. The influence of several factors, including solvent type, particle size, temperature, and solid-to-solvent ratio, on the extraction efficiency was investigated. The results indicated that smaller particle size significantly improved β-carotene extractability. Among acetone, hexane, and ethyl acetate, ethyl acetate proved to be the most effective solvent for β-carotene extraction. At a solvent-to-material ratio of 10:1, the highest β-carotene recovery was attained after three consecutive extractions, with the first extraction accounting for 69.9% of the total recovery. A kinetic model incorporating the effects of driving force and degradation on the kinetics of β-carotene extraction was developed. The calculated activation energies for extraction and degradation were determined to be 9.15 and 69.71 kJ/mol, respectively. This study demonstrates the potential of β-carotene extraction from gac peel and provides valuable insights into the underlying kinetics.

Key findings

- The influence of various factors, such as solvent type, particle size, temperature, and solid-to-solvent ratio, on the extraction of β-carotene was investigated.
- Pseudo-first order and pseudo-second order kinetic models were found to be unsuitable for describing β-carotene extraction because of the impact of β-carotene degradation.
- A pseudo-homogeneous kinetic model incorporating a linear driving force was developed, yielding valuable kinetic parameters.

Accompanying information

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Sustainable Development Goals

1. Introduction

Gac (*Momordica cochinchinensis* Spreng) fruit peel, often discarded as waste, has been recognized as a valuable source of bioactive compounds and antioxidants, including β-carotene [1]. β-carotene, a vital nutrient for humans and primarily recognized as provitamin A, is associated with a range of health benefits [2]. β-carotene's antioxidant properties and ability to enhance immune function contribute to disease prevention and overall well-being [3]. Hoe et al. [4] presented a scalable and inexpensive setup for the β-carotene extraction. Given its significant level of β-carotene [5], gac peel is a promising feedstock for recovering this valuable compound.

The scope of β-carotene extraction continues to expand, targeting various biological sources and their derived materials. Kanda et al. [6] successfully extracted β-carotene from the microalga *Dunaliella salina* using liquefied dimethyl ether. Gungor et al. [7] recovered β-carotene from pumpkin peel, achieving a hexane extractability of 125.75 mg/100 g of a sample. Jayesree et al. employed a water-induced hydrocolloidal complexation method to extract β-carotene from carrot peel, obtaining an efficiency of 1.17 mg/100 g of the sample [8]. Chifomboti and Chimphango [9] utilized physical methods to extract lipophilic β-carotene compounds from pumpkin peel; however, the raw extract contained oil and other constituents, necessitating a subsequent separation step. Recently, Hladnik et al. [10,11] extracted β-carotene from the yeast *Rhodotorula glutinis* using various solvents, with results indicating significantly high extraction efficiency achieved with ethyl acetate. Ludwig et al. [12] reported that the recovery of β-carotene from *Dunaliella salina* using hexane as a solvent is less energy-intensive than using supercritical CO_2 extraction methods.

For β-carotene extraction, common solvents include acetone, hexane, and ethyl acetate. Hagos et al. [13] demonstrated the potential of an acetone solvent for extracting β-carotene from pumpkin (*Cucurbita maxima*). Sun et al. [14] found that ethyl acetate can extract approximately 1.5 times more β-carotene from citrus peels than hexane. These findings highlighted the crucial role of solvent selection in β-carotene extraction from various plant materials.

Various mathematical models, including pseudo-first order (PFO), pseudo-second order (PSO), diffusion, and Weibull models, were employed to study extraction kinetics [15–17]. Several authors developed kinetic models that account for degradation during extraction [18, 19]. While Subra et al. [20] and Döker et al. [21] developed kinetic models for β-carotene extraction, they did not consider its degradation. However, studies by Sun et al. [14] and Takahashi et al. [22] indicated that β-carotene can be degraded in organic solvents. In sunflower oil, the model of β-carotene thermal degradation was developed by Kumar et al. [23]. The degradation of β-carotene needs to be considered in modeling the β-carotene extraction [24]. To the best of our knowledge, no published kinetic models for batch β-carotene extraction have yet incorporated degradation.

In this study, β-carotene was recovered from gac peel using a conventional solid-liquid extraction method. The influence of various factors on the extraction process, including solvent type (acetone, hexane, and ethyl acetate), liquid-to-solid ratio (3:1, 5:1, 10:1, 15:1, 20:1, and 30:1 mL/g), temperature (303, 331, and 340 K), and particle size (0.083±0.028, 0.169±0.016, and 2.031±0.113 mm), was investigated. A kinetic model incorporating degradation and driving force was developed to gain insights into the thermodynamic properties of the extraction process.

2. Experimental

2.1. Material, chemicals, and apparatus

Twenty fresh ripe gac fruits were collected from a local market, yielding a total wet peel weight of 25 kg.

Hexane (99.6% w/w), acetone (99.5% w/w), and ethyl acetate (99.7% w/w) were supplied by Xilong Scientific Co. Ltd (China). Standard β-carotene (96% w/w) was purchased from Shanghai Macklin Biochemical Co. Ltd.

UV-Vis spectra were recorded in a range of 200–800 nm using an Evolution 350 spectrometer (ThermoFisher Scientific, USA).

Computrac MAX 5000XL was operated at 105 °C for determing moisture content of the material (according to the AOCS Ca 2c-25 method).

2.2. Preparation of raw material

Gac fruits were washed with tap water and then halved. The peels were cut into pieces with dimensions of 1×1×5 cm (width × height × length). The peels were dried at 373 K until the moisture content reached 10–15%. Dried peels were crushed into small particles, mixed thoroughly, and sieved into different size ranges using 0.15, 1, and 2.38 mm sieves. The particles were stored in closed glass containers at room temperature.

Raw samples were photographed using a Rievbcau digital microscope with HiView software. ImageJ software was used to analyze particle size distribution based on these photos.

2.3. Batch extraction

Batch extraction of β-carotene from gac peel was performed in a three-necked round-bottom flask (500 mL) containing 300 mL of solvent with a condenser. The mixture was stirred, and temperature was monitored using a glass alcohol thermometer. The effects of particle sizes, solvents (acetone, hexane, and ethyl acetate), solvent-to-material ratios (3:1, 5:1, 10:1, 15:1, 20:1, 30:1 mL g^{-1}), and temperatures (303, 331, and 340 K) were investigated. At predetermined intervals (5, 10, 15, 20, 30, 40, and 60 min), 2 mL of sample was withdrawn, quickly filtered, diluted, and analyzed for β-carotene concentration. The experiments were conducted three times for each condition and performed under atmospheric pressure (1 atm).

2.4. Analysis of β-carotene concentration

Stock β-carotene solutions (500 mg L^{-1}) were prepared by dissolving 52.1 mg of standard β-carotene (96% w/w) in 100 mL of different solvents. These stock solutions were subsequently diluted with the corresponding solvents to create working solutions with β-carotene concentrations at

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0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 mg L^{-1}. The absorbance of these working solutions at 450 nm was determined to generate calibration curves.

2.5. Determination of β-carotene content in material

The content of β-carotene in the material was determined following the protocol established by Biswas et al. [25]. The dried peel was ground using a mortar and pestle, subsequently sieved through a 0.075-mm mesh to obtain a fine powder. One gram of the resulting powder was placed in a 50-mL Erlenmeyer flask with a stopper, where it was mixed with 10 mL of ethyl acetate under dark conditions. The mixture was magnetically stirred for 30 min before being transferred to a 50-mL polypropylene screw-cap tube for centrifugation at 4,000 rpm for 1 min. The supernatant was then decanted into a 500-mL volumetric flask, while the remaining solid was returned to the Erlenmeyer flask and extracted repeatedly until the extract became colorless. Ethyl acetate was added to the volumetric flask to reach the calibration mark. The final solution's absorbance was measured at 450 nm to calculate the concentration of β-carotene following the calibration curve as presented in Section 3.1. All measurements for determining β-carotene content in the material were performed in triplicate.

3. Results and Discussion

3.1. UV-Vis spectra of β-carotene in different solvents

β-carotene's delocalized π-electrons interact with solvents to varying degrees, influencing absorption peak intensities [26]. Figure 1a shows the UV-Vis spectra ranging from 350 to 650 nm of standard β-carotene in acetone, ethyl acetate, and hexane at 5 mg L^{-1}. All spectra exhibit a similar characteristic shape with three distinct visible peaks, likely due to the transition of electronic states in double bonds of β-carotene's structure [27]. This results in strong absorption at 424, 453, and 481 nm, as reported in previous findings [28]. The shape and peak positions of the UV-Vis spectrum are critical indicators of β-carotene's presence in the extracts.

UV-Vis spectroscopy is a valuable technique for β-carotene determination due to its simplicity, ease of use, and affordability [29, 30]. As shown in Figure 1a, the most substantial peaks in UV-Vis spectra appear at 450 nm. Consequently, the absorbance at this wavelength was used to construct the calibration curves, represented by Equations (i)-(iii) in Table S1. These equations depict the linear relationships between peak intensity and β-carotene concentration (0.5–5 mg L^{-1}) observed in acetone, hexane, and ethyl acetate (Figure 1b) with the closeness of R^2 to 1.

The absorbance intensity of β-carotene varies with the solvent, as indicated by its molar absorptivity. Carft's study [31] revealed that the molar absorptivity of β-carotene follows the order of hexane > acetone > ethyl acetate, leading the peak height at 450 nm to follow this trend. The results presented in Figure 1a align well with this observation.

The UV-Vis spectra of extracts from gac peel extraction using acetone, hexane, and ethyl acetate solvents, as depicted in Figure S1, closely resemble the UV-Vis spectra of standard β-carotene solutions (Figure 1a). This result demonstrates that UV-Vis spectroscopy can effectively identify and quantify β-carotene from gac peel extracts.

3.2. Study on β-carotene extraction

3.2.1. Effect of particle size

Particle size significantly influences β-carotene extraction from gac peel. Figure S2 illustrates the particle size distribution curves for three size ranges: 1–2.5 mm, 0.15–1 mm, and <0.15 mm. The average particle sizes were determined to be 0.083±0.028, 0.169±0.016, and 2.031±0.113 mm, respectively. A smaller particle size increases the interfacial area and enhances solvent penetration into the gac peel matrix, thereby enhancing extraction yield [32].

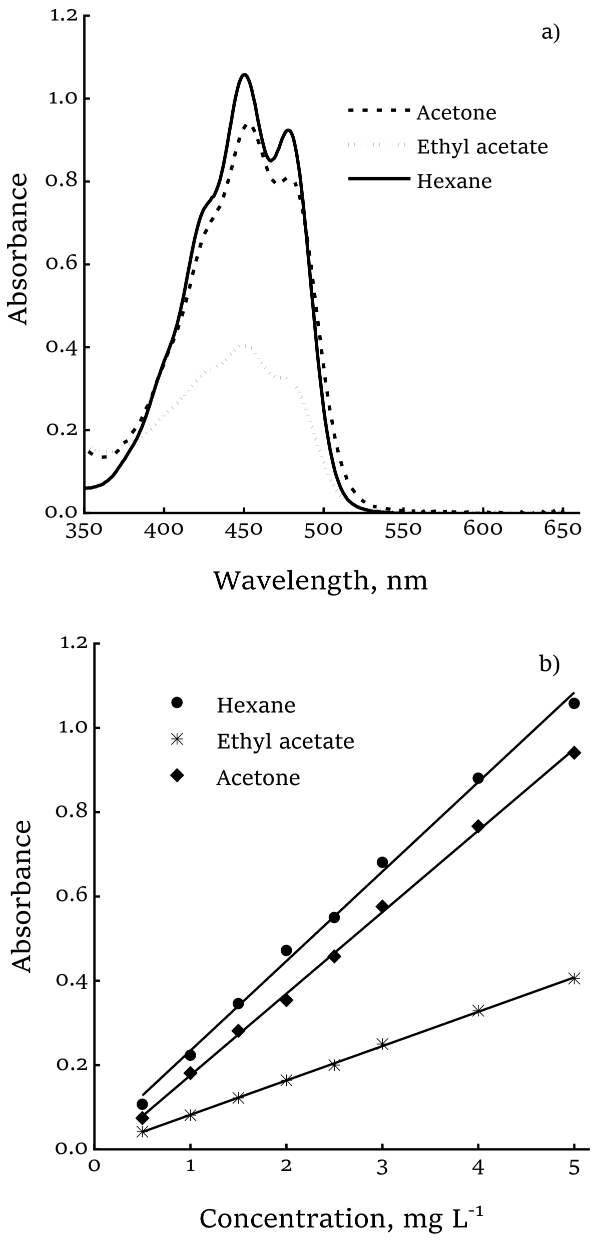

Figure 1 Effect of different solvents on a) UV-Vis spectra and b) calibration curves of β-carotene.

To evaluate the impact of particle size on β-carotene extraction, experiments were conducted using acetone at a solvent-to-material ratio of 5:1 and a temperature of 303 K. Smaller particles offer a larger surface area and a shorter diffusion path for the solvent, facilitating β-carotene extraction compared to larger particles [14, 32]. As illustrated in Figure 2, β-carotene concentration in the extract increases with decreasing particle size. Particles in the 1-2.5 mm range yielded β-carotene concentrations approximately 2 times lower than those in smaller particle sizes. This trend aligns with previous research findings [14, 33]. Consequently, particles smaller than 0.15 mm were used in subsequent experiments.

3.2.2. Effect of solvents

Acetone, hexane, and ethyl acetate were used to extract β-carotene from gac peels within 60 minutes at 303 K with a solvent-to-material ratio of 5:1. As illustrated in Figure 3, β-carotene concentration in ethyl acetate extract was significantly higher than with acetone or hexane, consistent with previous research [14, 34]. While polarity is a crucial factor in understanding solvent-β-carotene interactions [35], its precise role in this study remains unclear. Although hexane, a non-polar solvent, generally offers better solubility for β-carotene than ethyl acetate or acetone [31], it may not interact effectively with β-carotene's structure, resulting in lower β-carotene extractability. Conversely, ethyl acetate and acetone molecules contain carboxyl (–COOH) and carbonyl (–C=O) groups, respectively, which can form hydrogen bonds with –CH groups in β-carotene molecule [36], facilitating β-carotene extraction from the gac peel matrix. With two electronegative oxygen atoms, ethyl acetate is likely to exhibit stronger interactions with β-carotene compared to acetone. Additionally, β-carotene exhibits higher solubility in ethyl acetate than in acetone. The synergistic advantage of ethyl acetate for β-carotene extraction makes it the preferred solvent for future experiments.

3.2.3. Effect of solvent-to-material ratio

The solvent-to-material ratio is a crucial factor in β-carotene extraction from gac peel. To maximize β-carotene recovery, the raw material was subjected to three consecutive extractions at the desired solvent-to-material ratios at a temperature of 303 K over 60 minutes. The extracted amounts and concentrations of β-carotene at various solvent-to-material ratios and extraction times are presented in Figure 4.

As shown in Figure 4a, the extracted mass of β-carotene per gram of used material gradually decreased with increasing extraction cycles at each solvent-to-material ratio. This is attributed to the reduced amount of extractable β-carotene in the material over-extraction time. For the first extraction, the extracted β-carotene increased with increasing solvent-to-material ratio from 3:1 to 10:1, reaching a plateau at ratios beyond 15:1. Although a higher solvent volume increases the extraction driving force, the extractable β-carotene remains finite [37, 38]. For the second and third extractions, β-carotene yield showed minimal fluctuation. This trend differs from previous observations for β-carotene extraction from wet *Tetradesmus obliquus* using methanol [39], where the biomass was hydrated in the first extraction, causing a positive effect for β-carotene extraction as contacting pure solvent in the second extraction.

As depicted in Figure 4b, a 10:1 ratio achieved the highest β-carotene yield from gac peel. Consequently, this ratio was adopted for subsequent extraction runs, yielding a total of 1.56 g of β-carotene obtained after three times of extraction. With β-carotene content in material determined to be 1.72 ± 0.03 mg/g, this result corresponds to a recovery efficiency of approximately 92.1%. The first extraction accounted for approximately 69.9% in three times of extraction. While using equivalent amounts of solvent, the second and third extractions yielded negligible amounts of β-carotene, leading to increased solvent utilization.

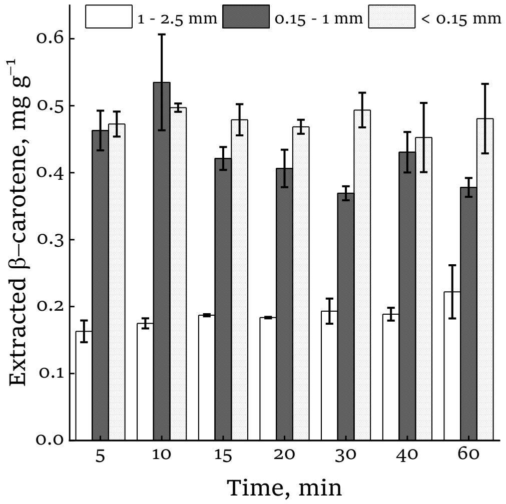

Figure 2 Effect of particle size on the β-carotene concentration in extracts.

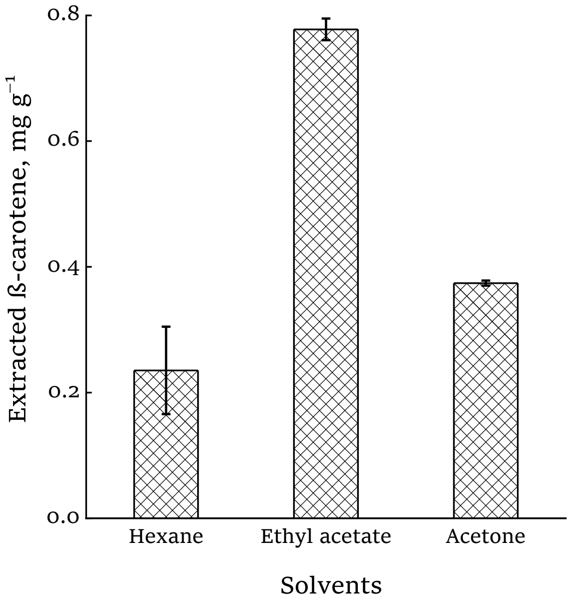

Figure 3 Effect of solvent on the β-carotene concentation in extracts.

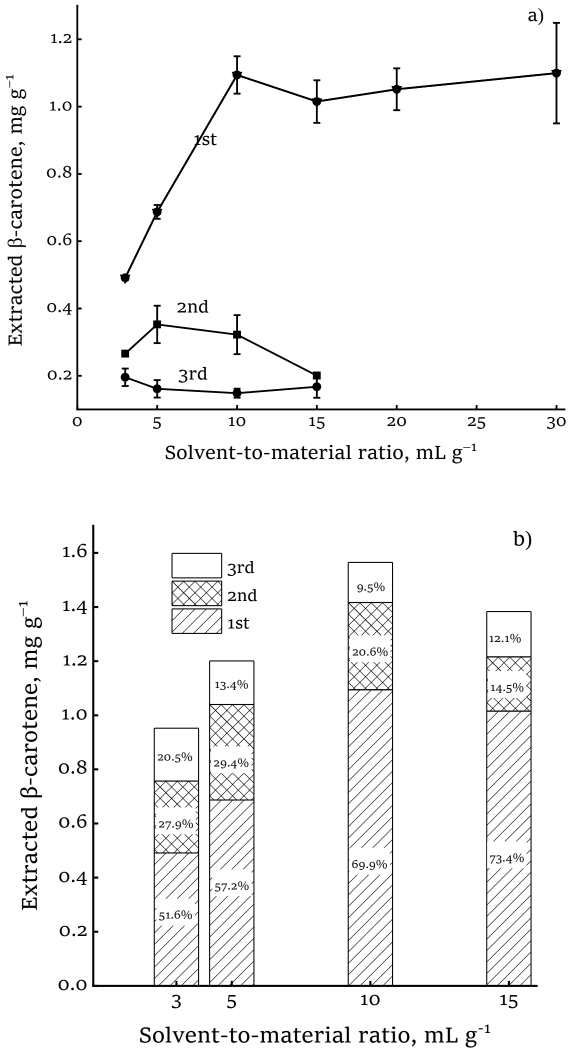

Figure 4 Effects of different solvents on a) β-carotene yield, b) relative extracted β-carotene over three consecutive extractions.

3.3. Study on kinetic and thermodynamics of β-carotene extraction

3.3.1. Notes on β-carotene extraction

Stefanovich and Krel [40] reported that β-carotene is susceptible to degradation over time. Our study confirmed this observation through a straightforward experiment. Five glass screw-cap tubes containing 10 mL of a β-carotene solution in ethyl acetate at an initial concentration of 40 mg/L were purged with nitrogen gas for 5 min to remove oxygen and then were stored at 303 K. At 1-hour intervals, one tube was sampled to measure the remaining concentration of β-carotene. This protocol was again performed for a storage temperature of 333 K.

As presented in Figure S3, β-carotene degradation accelerates at higher temperatures. Consequently, the degradation of β-carotene must be considered in the kinetic analysis of β-carotene extraction, leading to three distinct stages:

- Initial stage: the extraction rate exceeds the degradation rate, increasing the β-carotene concentration in the extract.
- Intermediate stage: the degradation rate surpasses the extraction rate, causing a decrease in β-carotene concentration in the extract.
- Equilibrium stage: the extraction rate equals the degradation rate, resulting in a maximum β-carotene concentration in the extract.

Previous studies successfully employed pseudo-first order (PFO) and pseudo-second order (PSO) models to describe the kinetics of extraction processes [15, 41]. However, these models assume a direct correlation between extraction rate and driving force, limiting their accuracy to represent the maximum concentration observed in this study (Appendix S1, S2). To address this limitation, a novel kinetic model tailored for β-carotene extraction is required to provide a more comprehensive understanding of the process.

3.3.2. Proposal of a new kinetic model

A general equation for the time-dependence of β-carotene in extract follows Equation 1:

$$\frac{dC}{dt} = r_\mathrm{e} - r_\mathrm{d}\,, \tag{1}$$

where r_e and r_d represent the extraction rate and degradation rate, respectively. Based on the chemical reaction rate law, $r_\mathrm{d} = k_\mathrm{d}C$, Equation 1 can be expressed as follows:

$$\frac{dC}{dt} = r_\mathrm{e} - k_\mathrm{d}C\,, \tag{2}$$

where k_d represents the degradation rate constant.

A pseudo-homogeneous kinetic model incorporating linear driving force (PHK-LDF) was employed to investigate the influence of solvent on β-carotene extractability. In this approach, the extraction rate is assumed following the rate law of a chemical reaction, which is given by Equation 3:

$$r_\mathrm{e} = -\frac{dC_\mathrm{s}}{dt} = k_\mathrm{e}C_\mathrm{s}h\,, \tag{3}$$

where h represents the driving force for β-carotene extraction, which is assumed to be the difference between the equilibrium concentration of β-carotene in the solvent (C_eq) and its concentration at time t (C), $h = C_\mathrm{eq} - C$, k_e is the extraction rate constant, and C_s represents the apparent concentration of β-carotene. Equation 3, incorporating this driving force, is presented as Equation 4:

$$\frac{dC_\mathrm{s}}{dt} = -k_\mathrm{e}C_\mathrm{s}\left(C_\mathrm{eq} - C\right). \tag{4}$$

Therefore, Equation 2 can be rewritten as Equation 5:

$$\frac{dC}{dt} = -k_\mathrm{e}C_\mathrm{s}\left(C_\mathrm{eq} - C\right) - k_\mathrm{d}C\,. \tag{5}$$

The system of Equations 4 and 5 can be numerically solved using a fourth-order Runge-Kutta method with initial conditions (t = 0) as presented by Equation 6:

$$\begin{cases} C_s(0) = C_{s,\max} \\ C(0) = 0 \end{cases}, \tag{6}$$

where $C_{s,\max}$ corresponds to the maximum apparent concentration, representing the total β-carotene content per volume of solvent.

3.3.3. Validation of the proposed kinetic model

To validate the PHK-LDF model, experimental data from β-carotene extractions at different temperatures (303, 331, and 340 K) using ethyl acetate at a solvent-to-material ratio of 10:1 was regressed against the model. As shown in Table S2, the PHK-LDF model fits the experimental data well, with high R^2 values at each temperature. Both the extraction rate constant (k_e) and the degradation rate constant (k_d) increase with temperature, and k_e consistently exceeds k_d. Figure 5a illustrates the experimental data points along with the corresponding fitting curves generated by the PHK-LDF model.

The activation energies (E_a) for β-carotene extraction and degradation were determined using Arrhenius's law. A linear relationship between ln*k* and 1000/*T* (Figure 5b) provided the slope, proportional to $-E_a/R_g$ (R_g is the ideal gas constant) [42]. The calculated E_a for degradation (69.71 kJ mol^{-1}) was 7.6 times higher than that for extraction (9.15 kJ mol^{-1}). This result demonstrates that while both extraction and degradation rates become faster at higher temperatures, degradation is far more temperature-sensitive than extraction, explaining why the β-carotene concentration in the extracts at 331 and 340 K differs only slightly (Figure 5a).

4. Limitations

We have not yet addressed solvent recovery or the subsequent purification of the extracted β-carotene. Future work should explore methods for solvent recycling and refining the extract to obtain high-purity β-carotene. Furthermore, evaluating the biochemical composition of the final extract in more detail, as well as exploring integrated evaporation of by-products, could also help overcome current limitations.

5. Conclusions

In this study, β-carotene was successfully recovered from gac peel using a conventional solid-liquid extraction method. The effects of various factors, namely solvents (hexane, acetone, and ethyl acetate), particle size, solvent-to-material ratio, and temperature, was investigated. The results showed that β-carotene extractability is strongly influenced by solvent choice, following the order of ethyl acetate > acetone > hexane. Within the tested particle size range, smaller particles yielded higher extraction efficiency. The highest total β-carotene mass was achieved at a ratio of solvent volume to material of 10:1 (mL g^{-1}). Approximately 70% of the total β-carotene was extracted during the first of three consecutive extraction cycles. Kinetic analysis revealed that the PFO and PSO models were inadequate for describing β-carotene extraction from gac peel, likely due to β-carotene's thermo-degradable nature. A PHK-LDF model, incorporating degradation and driving force, was developed and fitted well to the experimental results. The activation energy for degradation (69.71 kJ mol^{-1}) was significantly higher than for extraction (9.15 kJ mol^{-1}), confirming the temperature-sensitivity of the β-carotene degradation. This study provides valuable kinetic and thermodynamic insights, supporting future industrial-scale applications where the solvent can be fully recovered and reused.

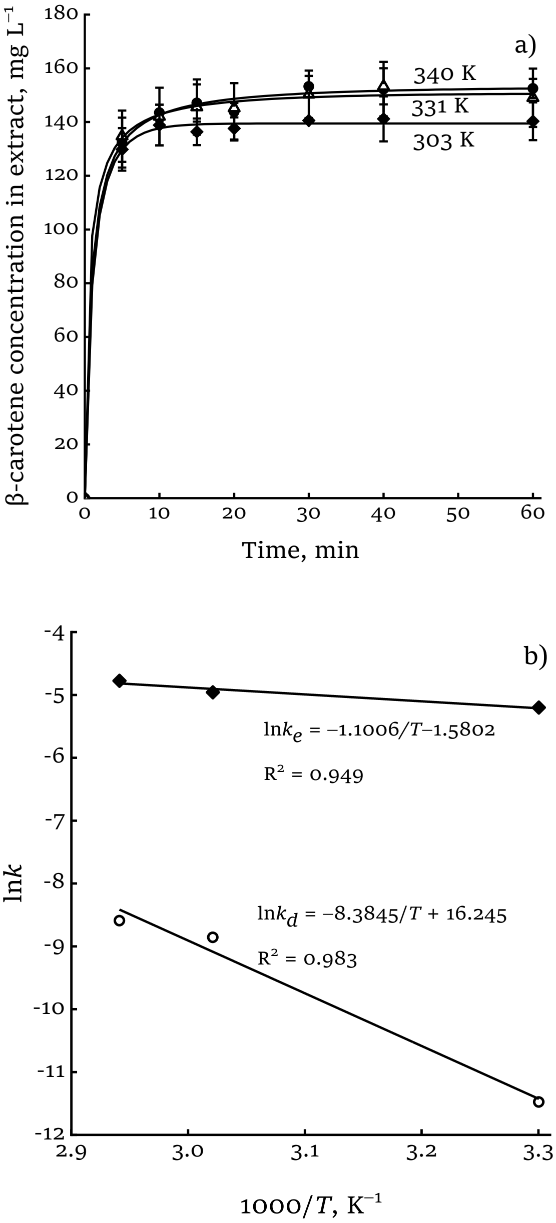

Figure 5 Variation of β-carotene concentration in extract again time at different temperatures (Experimental result: discrete points, simulation: solid curve) (a), plots of ln*k* and 1000/*T* (b).

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Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

Data availability statement

Data will be made available on request.

Acknowledgments

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Conceptualization: T.D.P.Q., H.D.T.
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Supervision: H.D.T.
Validation: D.H.T., U.P.N.T.
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Conflict of interest

The authors declare no conflict of interest.

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