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Research article

Green Extraction of Bioactive Compounds from Tomato Pomace Using Fatty Acid Ethyl Esters Derived from Krabok Seed Oil

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Abstract: Tomato pomace, a byproduct of food processing, is rich in bioactive carotenoids such as lycopene and β-carotene, which are valued for their antioxidant properties but typically extracted using toxic organic solvents. This study aimed to develop an environmentally friendly approach for extracting and quantifying these compounds using fatty acid ethyl esters (FAEE) derived from Krabok seed oil, in comparison with hexane. Conventional maceration extraction (ME) and ultrasonic-assisted extraction (UAE) were applied, and the extracts were analyzed for lycopene and β-carotene content, antioxidant activity, and storage stability. The results showed that the UAE was more efficient than ME, while hexane provided higher extraction yields due to its lower viscosity, with lycopene and β-carotene contents of 67.97–75.33 and 10.51–11.50 mg/100 g dry weight (DW), respectively. FAEE extraction yielded slightly lower amounts (59.92–63.60 mg/100 g DW lycopene and 1.63–7.01 mg/100 g DW β-carotene) but exhibited superior antioxidant activity, with DPPH inhibition up to 92.05% when combined with the extracted compounds. Stability tests further revealed that FAEE-extracted carotenoids were more resistant to degradation, retaining up to 90% lycopene and 85% β-carotene after 56 days, whereas hexane-extracted compounds degraded rapidly. Overall, FAEE demonstrates strong potential as a sustainable, non-toxic solvent for the recovery of bioactive carotenoids from tomato pomace, supporting applications in the food and cosmetic industries.

Keywords: Fatty acid ethyl esters (FAEE); green technique; lycopene; β–Carotene; antioxidant activity

1. Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) are a significant economic and industrial crop in Thailand, widely consumed across the country. Sakon Nakhon Province leads the Northeast region in tomato production, with a total cultivation area of 5,232 rai. Tomatoes are rich in carotenoids, primarily lycopene

and β−carotene, which are red−orange pigments. Lycopene, abundant in red tomatoes, serves as a potent antioxidant. β−carotene, predominantly found in orange tomatoes, is a precursor for vitamin A synthesis, which is essential for maintaining healthy vision [1]. In addition to carotenoids, tomatoes are an excellent source of vitamins and minerals beneficial to human health, including vitamin C, vitamin A, and vitamin E [2]. Vitamin E, in particular, functions as an antioxidant, playing a critical role in supporting liver health and blood functions while combating free radicals [3]. Fresh tomatoes are commonly processed into products like juice and sauce, generating by−byproducts such as skins, peels, and seeds, which account for 10–15% of the fresh weight of the original tomatoes. These residual tomato pomace are typically extracted through maceration or ultrasonic extraction. In maceration, bioactive compounds are extracted by immersing the plant material in a suitable solvent, allowing passive diffusion of solutes across cell walls, followed by filtration and squeezing. While this method avoids heat degradation, it is solvent-intensive and time-consuming. In contrast, ultrasonic-assisted extraction relies on acoustic cavitation, where alternating high- and low-pressure sound waves (20–100 kHz) generate microbubbles that collapse violently, disrupting cell walls and enhancing solvent penetration. This accelerates mass transfer, reduces solvent usage, and improves extraction efficiency. Common solvents include water or organic solvents like hexane, which, while effective for non-polar compounds, pose environmental and health risks due to their volatility [5–6].

Many previous studies have highlighted the use of environmentally friendly extraction methods in place of chemicals. For instance, Sharma et al. [7] investigated the valorization of seabuckthorn pomace for extracting bioactive carotenoids using green extraction techniques. They applied ultrasonic and microwave−assisted extractions combined with green solvents like edible oils, aiming to improve extraction efficiency while minimizing environmental impact. This study advocates for sustainable methods to recover valuable carotenoids from by-products, offering an eco-friendlier alternative to conventional extraction processes in the food and pharmaceutical industries. Yara−Varón et al. [8] explored the use of vegetable oils as eco−friendly solvents for extracting and developing food and natural products. Oils like olive and coconut oil effectively and safely extract compounds such as phenols and carotenoids, reducing reliance on toxic synthetic solvents. This sustainable approach minimizes environmental impact and enhances the industrial value of vegetable oils. Despite challenges in cost and efficiency, it presents a promising alternative for safe, sustainable production. Diacon et al. [9] investigated fatty acid ethyl esters (FAEE) as a green solvent for extracting carotenoids from tomato waste. FAEE achieved up to 90% carotenoid recovery compared to hexane, reducing toxic chemical use and promoting sustainable practices. This efficient process adds value to agricultural waste and aligns with the goals of the circular economy.

The research report above highlights that vegetable oils and their derivatives, such as FAEE, are gaining attention as solvents for extracting bioactive carotenoids. This approach offers a promising alternative for developing environmentally friendly and safe green solvents for applying extracts in various fields. From the previous research report of our research group, it was found that the oil from Krabok seed (*Irvingia malayana*) contains major fatty acids, with lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) making up approximately 52% and 35% by weight, respectively. This places it in the category of oils with small molecules. Additionally, the oil demonstrates important chemical properties, including high oxidation stability and a low acid number [10]. Additionally, our research on the synthesis of FAEE from Krabok seed oil demonstrated its excellent potential for use in herbal massage oil. The process achieved a high yield of 99.83 ± 0.17% under optimal conditions. The FAEE primarily contained lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$), with 97 wt.% saturated fatty acids. Its physicochemical properties included a viscosity of 1.73 ± 0.03 cSt/s, density of 0.8199 ± 0.0049 g/cm^3, cloud point of +4 °C, and pour point of −1 °C. Additionally, it exhibited good acidity and oxidation stability, making it a sustainable and promising ingredient for cosmetic applications [11]. The low viscosity of FAEE enables better penetration into plant matrices, thereby enhancing mass transfer and improving extraction efficiency. Its high oxidation stability also prevents degradation of sensitive carotenoids during extraction. Moreover, as it is derived from renewable sources and biodegradable, FAEE aligns with the principles of green chemistry, offering a safer and more sustainable alternative to conventional organic solvents such as hexane.

Although previous studies have explored various green solvents and extraction methods for recovering carotenoids from plant by-products, there is limited research on the use of FAEE derived from locally available Krabok seed oil as an eco–friendly solvent for tomato pomace. Therefore, this study investigates extraction techniques and quantification of bioactive compounds from tomato pomace, a waste byproduct of processing, employing environmentally sustainable methods. Solvent extraction methods, including conventional maceration and ultrasonic–assisted extraction, were utilized. FAEE derived from the transesterification of Krabok seed oil was evaluated as a solvent and compared to hexane, given that both lycopene and β-carotene are non–polar and fat–soluble. FAEE demonstrated efficacy as a solvent. The bioactive compounds, specifically lycopene and β–carotene, were quantified using ultraviolet–visible spectroscopy. Additionally, antioxidant activity was assessed using the DPPH assay, providing critical insights for optimizing extraction techniques and laying the groundwork for potential applications in food or cosmetic products.

2. Materials and Methods

2.1 Materials

The tomato pomace was sourced from the Royal Food Processing Factory No. 3, located in Tao Ngoi Subdistrict, Tao Ngoi District, Sakon Nakhon Province, Thailand. The preparation process involved drying the pulp at 50 °C for 8 h, followed by fine grinding and sieving. The processed pulp was then stored in a −40 °C freezer to maintain its quality and prevent mold growth by avoiding exposure to humidity. Fatty acid ethyl esters (FAEE) derived from the transesterification of Krabok seed oil were obtained from the Biomass Energy Laboratory, Research and Development Institute, Sakon Nakhon Rajabhat University, Thailand. The physicochemical properties of FAEE were investigated and reported in the study by Thangthong et al. [11]. The main chemicals used in this research included hexane (C_6H_{14}), ethanol (C_2H_5OH), acetone (C_3H_6O), 2,2–diphenyl–1–picrylhydrazyl (DPPH), lycopene standard ($C_{40}H_{56}$), and β–carotene standard (β–carotene; $C_{40}H_{56}$). All chemicals were of AR grade and sourced from RCI Lab Scan, QREC, Aldrich, and Sigma–Aldrich brands.

2.2 Study of the Efficiency of Extraction Techniques

The study of the efficiency of bioactive compound extraction techniques in this research includes simple maceration extraction (ME) and ultrasonic–assisted extraction (UAE) using hexane and FAEE as solvents. For the simple maceration extraction, about 1 g of dried tomato pomace is weighed (with the exact weight recorded) and placed in a brown bottle. Then, 10 mL of hexane is pipetted into the bottle, and the mixture is shaken using a shaker for 1 min. The bottle is then placed in a hot water bath at 50 °C for 60 min. These extraction temperatures and durations were selected to optimize carotenoid recovery while minimizing thermal degradation, as reported in previous studies [5–6]. Although carotenoids such as lycopene and β-carotene are heat-sensitive, these conditions have been shown to preserve the majority of compounds, ensuring reliable quantification. After the heating period, the solution is centrifuged at 3,000 rpm for 10 min. The resulting solution is stored in a brown bottle and kept in a refrigerator at −20 °C until further analysis. This process is repeated twice more. The same experiment is then conducted, but with FAEE replacing hexane as the solvent. The ultrasonic extraction process began by accurately weighing 1 g of dried tomato pomace (recording the exact weight) and placing it in a brown bottle. Next, 10 mL of hexane was added, and the mixture was shaken for 1 min using a shaker. The sample was then subjected to ultrasonic treatment in an ultrasonic cleaner at 50 °C and 40 kHz with an ultrasonic power of 120 W (power density ≈ 12 W/100 mL) for 60 min, following conditions reported in previous studies [5–7]. After ultrasonic-assisted extraction, the solution was centrifuged at 3,000 rpm for 10 min. The resulting supernatant was transferred to a brown bottle and stored in a refrigerator at −20 °C for further analysis. The experiment was repeated two more times under the same conditions. Subsequently, the procedure was repeated using FAEE as the solvent instead of hexane. All experiments were performed in triplicate (n = 3), and results are expressed as mean ± SD.

2.3 Determination of lycopene and β–carotene content in tomato pomace extracts

The analysis was performed following methods modified from Diacon et al. [9] and Fish [12]. The procedure began with creating an equation for calculating the amount of lycopene. A lycopene standard

solution was prepared by weighing 0.0050 g of lycopene standard (containing 6.4% lycopene), dissolving it in hexane, and diluting it to 50 mL in a volumetric flask to achieve a concentration of 6.4 mg/L. The standard solution was freshly prepared and used immediately. The subsequent steps involved repeating the process using FAEE as the solvent in place of hexane. A series of lycopene solutions with varying concentrations was then prepared by pipetting 1.5, 3.0, 4.5, 6.0, and 7.5 mL of the 6.4 mg/L lycopene standard solution into separate volumetric flasks and diluting each to 10 mL with hexane. This produced solutions with lycopene concentrations of 0.96, 1.92, 2.88, 3.84, and 4.8 mg/L, respectively. These solutions were used to construct standard curves at wavelengths of 450 and 503 nm. The experimental procedure was then repeated with FAEE as the solvent instead of hexane for comparative analysis.

The β−carotene standard solution was prepared by weighing 0.0050 g of β−carotene standard (containing 97.5% β−carotene), dissolving it in hexane, and adjusting the volume to 50 mL in a volumetric flask. This resulted in a β−carotene standard solution with a concentration of 97.5 mg/L, which was freshly prepared and used immediately. The experiment was repeated using FAEE as the solvent instead of hexane. The β−carotene solutions at various concentrations were then prepared as follows:

1) The standard graph at 450 nm (hexane and FAEE as solvents): The β−carotene standard solution (97.5 mg/L) was pipetted in volumes of 0.1, 0.2, 0.3, 0.4, and 0.5 mL, and the volume was adjusted to 10 mL with the respective solvent (hexane or FAEE). This yielded solutions with concentrations of 0.975, 1.95, 2.925, 3.9, and 4.875 mg/L.

2) the standard graph at 503 nm (hexane as solvent): The β−carotene standard solution was pipetted in volumes of 0.1, 0.5, 1.0, 1.5, and 2.0 mL, with the volume adjusted to 10 mL using hexane. The resulting concentrations were 0.975, 4.875, 9.75, 14.625, and 19.5 mg/L.

3) The standard graph at 503 nm (FAEE as solvent): The β−carotene standard solution was pipetted in volumes of 0.1, 0.3, 0.5, 0.7, and 0.9 mL, and the volume was adjusted to 10 mL using FAEE. This produced solutions with concentrations of 0.975, 2.925, 4.875, 6.825, and 8.775 mg/L.

These prepared solutions were used to construct β−carotene standard graphs at the specified wavelengths for comparative analysis between hexane and FAEE solvents. All prepared solutions were analyzed for absorbance using a UV−Visible spectrophotometer at the specified wavelengths of 450 and 503 nm. The measured absorbance values were used to construct standard curves by plotting absorbance against concentration (mg/L). Linear equations were derived from each curve, and the absorptivity values (ε) were calculated. These absorptivity values were then applied to the following equations for further analysis:

$$A_{503} = A_{lyc\text{-}503}\cdot[lyc] + A_{\beta\text{-}car\text{-}503}\cdot[\beta - car] \quad (1)$$

$$A_{450} = A_{lyc\text{-}450}\cdot[lyc] + A_{\beta\text{-}car\text{-}450}\cdot[\beta - car] \quad (2)$$

In addition, the concentration ranges selected for the calibration curves were justified based on preliminary assays and the expected analyte concentrations in the tomato extracts after applying the dilution factors. For lycopene, the calibration range (0.96–4.80 mg/L) was sufficient to cover the calculated concentrations of the extracts, which were approximately 1.75 mg/L for hexane extracts (df = 40) and 2.80 mg/L for FAEE extracts (df = 25), based on a lycopene content of ~70 mg/100 g DW. For β-carotene, the calibration ranges (0.975–4.875 mg/L at 450 nm; 0.975–19.5 mg/L at 503 nm; and 0.975–8.775 mg/L in FAEE) were also appropriate, although lower concentrations in some extracts (e.g., ~0.28 mg/L for hexane) required adjusting dilution factors to ensure that the measured concentrations fell within the validated linear ranges. Thus, the selected calibration ranges ensured accurate quantification while maintaining linearity and sensitivity.

In this study, the absorbance values at wavelengths of 503 nm and 450 nm, denoted as A_{503} and A_{450}, respectively, were measured. The absorptivity coefficients for lycopene and β−carotene at these wavelengths were also determined. Specifically, $A_{lyc\text{-}503}$ represents the absorptivity of lycopene at 503 nm, while $A_{lyc\text{-}450}$ represents the absorptivity of lycopene at 450 nm. In this study, the absorptivity coefficients are expressed in liters per milligram (L/mg) because the sample concentration was calculated in mg/L and the path length of the cuvette used in the UV−visible spectrophotometer was 1 cm. Therefore, the path length term (cm^{-1}) in the

conventional unit (L/mg·cm) is omitted, as it equals unity under these experimental conditions. Similarly, $A_{\beta\text{-car-503}}$ and $A_{\beta\text{-car-450}}$ represent the absorptivity of β−carotene at 503 nm and 450 nm, respectively. Using these values, the concentrations of lycopene and β−carotene, denoted as [lyc] and [β−car] (in mg/L), were calculated by substituting the absorbance and absorptivity values into the corresponding equations. This approach ensured accurate quantification of the pigments based on their distinct spectral characteristics.

The determination of lycopene and β−carotene content in tomato pomace samples extracted using various techniques was carried out by measuring the absorbance of the sample solutions with a UV−Visible spectrophotometer at wavelengths of 450 nm and 503 nm. The recorded absorbance values were then used to calculate the concentrations of lycopene and β−carotene in mg/L using the equations derived from the standard curves. Finally, the amounts of lycopene and β−carotene were reported in terms of mg per 100 g of dry weight (mg/100 g DW) using the following equations:

$$\text{Lycopene or β−carotene content (mg/100 g DW)} = [(C/1000) \times df \times (V/W)] \times 100 \quad (3)$$

Where: C is the concentration of lycopene or β-carotene in the sample solution (mg/L), df is the dilution factor of the sample, V is the volume of the extract used for the analytical measurement (mL), and W is the weight of the dry sample (g). The determination of lycopene and β−carotene content in tomato pomace samples extracted using these techniques was carried out in three replicates to ensure accuracy and reliability of the results. The absorbance measurements were taken for each replicate, and the concentrations of lycopene and β−carotene were calculated using the corresponding equations. The results were then averaged to obtain the final values for lycopene and β−carotene content in the samples. All experiments were performed in triplicate (n = 3), and results are expressed as mean ± SD.

2.4 Antioxidant activity test by DPPH method

The DPPH antioxidant activity assay was adapted from the method of Chu et al. [13], Preecharram et al. [14], and Phewphong et al. [15]. The experimental procedure began by preparing a 0.3 mM DPPH solution. This was done by weighing 0.0059 g of 2,2−diphenyl−1−picrylhydrazyl (DPPH radical), dissolving it in ethanol, and adjusting the volume to 50 mL in a volumetric flask (the solution should be prepared and used immediately). Next, a 5−fold diluted sample extract was prepared by pipetting 5 mL of the sample extract into a volumetric flask and adjusting the volume with hexane to 25 mL. The experiment was repeated as described, but the sample extract was replaced with FAEE. For the antioxidant activity test, 3 mL of the diluted sample solution was pipetted into a test tube, followed by the addition of 1.5 mL of DPPH solution. The solution was mixed by shaking for 10 seconds with a vortex mixer and then left in the dark for 15 min. The concentration of extracts used in the DPPH assay corresponded to a 5-fold dilution of the crude extracts, ensuring that absorbance values were within the measurable range of the assay. The absorbance of the solution was measured using a UV−Visible spectrophotometer at a wavelength of 540 nm. The absorbance values were then used to calculate the percentage of DPPH inhibition (% DPPH inhibition) using the following equation:

$$\%\ \text{DPPH inhibition} = [A_0 - (A - A_b)/A_0] \times 100 \quad (4)$$

Where: A_0 is the absorbance of the DPPH solution without the sample. A is the absorbance of the DPPH solution with the sample. A_b is the absorbance of the sample without DPPH. This equation measures the effectiveness of the sample in inhibiting the DPPH radical, reflecting its antioxidant activity. All experiments were performed in triplicate (n = 3), and results are expressed as mean ± SD.

2.5 Stability study of lycopene and β-carotene

The stability of lycopene and β-carotene in tomato pomace extracts obtained via ultrasound-assisted extraction (UAE) using FAEE as a solvent was evaluated over a period of 56 days. For the study, all extracts were stored in opaque amber glass containers to prevent light-induced degradation. The containers were tightly sealed to minimize oxygen exposure and evaporation. Samples were kept at a controlled temperature of 4 °C in a refrigerator with relative humidity below 60%. At predetermined intervals (0, 7, 14, 21, 28, 42, and 56 days), aliquots were taken to measure the concentrations of lycopene and β-carotene, as well as the DPPH

radical scavenging activity, following the procedures described in Section 2.4. These controlled storage conditions ensured reproducibility of the stability study. All experiments were performed in triplicate (n = 3), and results are expressed as mean ± SD.

2.6 Statistical analysis

The analysis of variance (ANOVA) was conducted using a one–way ANOVA to assess differences between experimental groups. Post–hoc comparisons were made using Duncan's multiple range test at a 95% confidence level. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software.

3. Results and Discussion

3.1 Physical characteristics of extracts from tomato pomace

The bioactive compounds were extracted from tomato pomace obtained from the Royal Project Food Factory 3, Tao Ngoi District, Sakon Nakhon Province. The tomato pomace was pretreated by baking at 50 °C for 8 h, followed by fine grinding and sieving. Two extraction techniques were employed: maceration extraction (ME) and ultrasonic–assisted extraction (UAE), using hexane and fatty acid ethyl esters (FAEE) as extraction solvents. The physical characteristics of the resulting extracts are presented in Figure 1(a) and (b). For ME, the extracts obtained with hexane as the solvent (tubes 1, 2, and 3) were observed as clear orange liquids. In comparison, extracts obtained using FAEE as the solvent (tubes 4, 5, and 6) exhibited a darker orange hue than those obtained with hexane. Similarly, the UAE method produced extracts with hexane (tubes 7, 8, and 9) and FAEE (tubes 10, 11, and 12) that displayed physical characteristics consistent with those obtained through the ME method. This suggests that the solvent type had a more pronounced effect on the coloration of the extracts than the extraction method employed. The darker orange hue observed in extracts obtained with FAEE is attributed to its higher solubilization capacity for non-polar carotenoids, thereby enhancing pigment extraction compared to hexane. FAEE itself exhibits a slightly pale yellow color, indicating that the observed coloration primarily reflects the concentration of extracted bioactive compounds rather than the solvent's inherent color. This suggests that solvent selection can significantly influence both the yield and apparent intensity of carotenoid–containing extracts. It is worth noting that the color evaluation in this study was based on visual observation. Quantitative colorimetric analysis, such as using the CIE Lab* color space, could provide a more objective comparison; however, visual assessment combined with measured carotenoid content provides a reasonable indication of extraction efficiency and solvent performance.

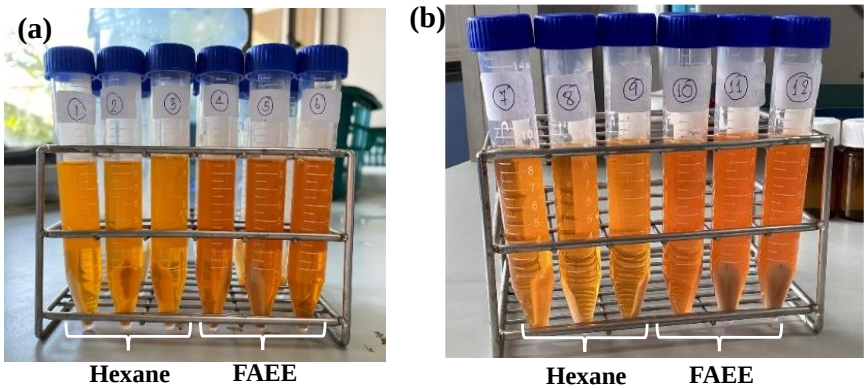

Figure 1. Physical characteristics of the extracts obtained using hexane and FAEE solvents: (a) maceration extraction (ME) and (b) ultrasonic–assisted extraction (UAE).

3.2 Development and validation of equations for lycopene and β–carotene quantification

Tomatoes are a rich source of carotenoids, particularly lycopene and β–carotene. These compounds are critical bioactive substances with overlapping absorption spectra, although their maximum absorption wavelengths (λ_{max}) differ. Accurate quantification of these compounds in tomato samples requires

consideration of their overlapping absorption. To address this, the relationship between the absorbance values at the λ_{max} of both compounds must be utilized for precise calculation, in accordance with Beer–Lambert's law. The law states that absorptivity is a constant specific to each substance under defined conditions, but can vary with changes in wavelength or external factors such as temperature and pH. This characteristic enables the formulation of equations for determining the concentrations of lycopene and β–carotene. The construction of these equations involved preparing standard solutions of lycopene and β–carotene at varying concentrations and analyzing their absorbance using a spectrophotometer at wavelengths of 450 nm and 503 nm, corresponding to their respective λ_{max}. Standard curves were generated, and the absorptivity values obtained from these curves, specific to the solvents used, were employed to develop equations for accurately quantifying lycopene and β–carotene in the samples [9, 12].

The quantification equation for lycopene and β–carotene in hexane was developed by analyzing standard solutions of these compounds at varying concentrations using Ultraviolet–Visible spectroscopy at wavelengths of 450 nm and 503 nm. The absorbance values and corresponding concentrations of the standard solutions were plotted to generate standard curves, as illustrated in Figure 2 (a), (b), (c) and (d). The results indicated that the standard curves for lycopene and β–carotene in hexane were linear, with coefficients of determination (R^2) ranging from 0.9973 to 1.0, demonstrating a strong linear relationship. The linear equations derived from these standard curves were used to calculate the absorptivity values, which are summarized in Table 1. In the case of hexane, the absorptivity values indicate that lycopene is more sensitive at 503 nm (0.1989 L/mg) than at 450 nm (0.1413 L/mg), whereas β-carotene is more sensitive at 450 nm (0.2540 L/mg) than at 503 nm (0.0402 L/mg). A higher absorptivity value implies greater sensitivity, meaning that small concentration changes produce more pronounced absorbance differences. This confirms that 503 nm is optimal for lycopene quantification, while 450 nm is preferred for β-carotene in hexane solvent. These absorptivity values serve as critical parameters for accurately determining the concentrations of lycopene and β–carotene in samples. The absorptivity values presented in Table 2 were substituted into equations (1) and (2), which led to the formation of equations (5) and (6) for lycopene and β–carotene, respectively. By combining these two equations, a unified equation was developed to calculate the concentrations of both compounds in hexane solvent. The resulting equations, as shown in Table 2, provide a comprehensive method for accurately quantifying lycopene and β–carotene based on their absorbance at the specified wavelengths.

$$A_{503} = 0.1989_{L/mg}\,[lyc]_{mg/L} + 0.0402_{L/mg}\,[\beta - car]_{mg/L} \quad (5)$$

$$A_{450} = 0.1413_{L/mg}\,[lyc]_{mg/L} + 0.254_{L/mg}\,[\beta - car]_{mg/L} \quad (6)$$

Table 1. Absorptivity values of lycopene and β–carotene in hexane and FAEE solvent.

Solvent	Wavelength (nm)	Absorptivity (L/mg)	
		Lycopene	β–Carotene
Hexane	503	0.1989	0.0402
	450	0.1413	0.2540
FAEE	503	0.2223	0.0997
	450	0.2256	0.2232

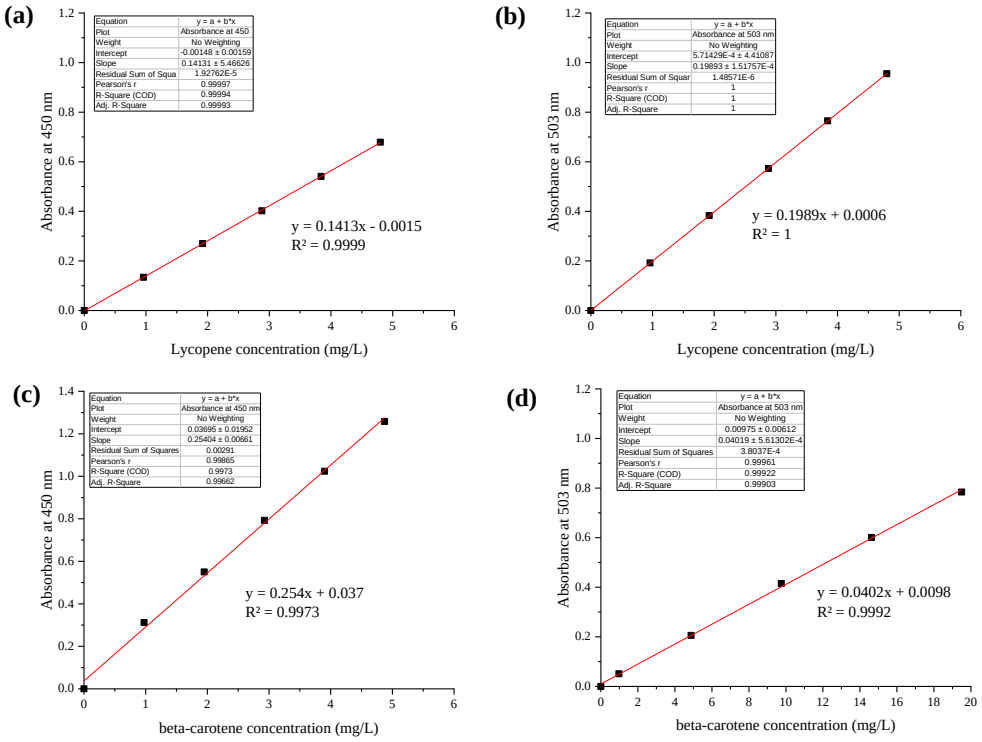

Figure 2. Standard curves for lycopene and β–carotene in hexane solvent: (a) lycopene at 450 nm, (b) lycopene at 503 nm, (c) β–carotene at 450 nm, and (d) β–carotene at 503 nm.

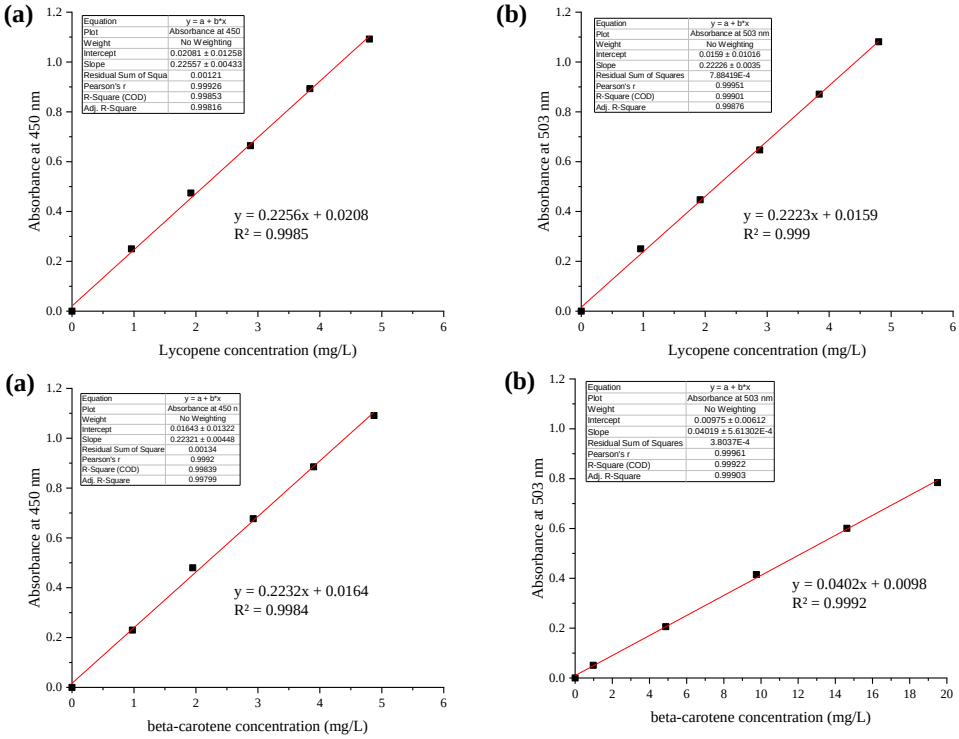

Figure 3. Standard curves for lycopene and β–carotene in FAEE solvent: (a) lycopene at 450 nm, (b) lycopene at 503 nm, (c) β–carotene at 450 nm, and (d) β–carotene at 503 nm.

Similarly, the calculation equations for lycopene and β−carotene content in FAEE solvent were developed by analyzing standard solutions of lycopene and β−carotene at various concentrations using UV−Visible spectroscopy at wavelengths of 450 nm and 503 nm. The absorbance values and corresponding concentrations of the standard solutions were used to generate standard curves, as shown in Figure 3 (a), (b), (c), and (d). The standard curves for both lycopene and β−carotene in FAEE solvent, measured at wavelengths of 450 nm and 503 nm, exhibited linear relationships with R^2 values ranging from 0.9984 to 0.9992. From these straight−line equations, the absorptivity values were determined, as presented in Table 1. When FAEE was used as the solvent, the absorptivity values of lycopene were relatively similar at 503 nm (0.2223 L/mg) and 450 nm (0.2256 L/mg), suggesting that either wavelength could be effectively employed. For β-carotene, absorptivity was also reasonably high at both wavelengths (0.0997 L/mg at 503 nm and 0.2232 L/mg at 450 nm), although 450 nm provided better sensitivity. These findings highlight solvent-dependent spectral characteristics that were subsequently incorporated into Equations (7) and (8), summarized in Table 2. These absorptivity values were then substituted into equations (1) and (2), resulting in equations (7) and (8), respectively. By combining equations (7) and (8), a unified set of equations for calculating the concentrations of lycopene and β−carotene in FAEE solvent was derived, as shown in Table 2. The experimental results demonstrated that the equations for determining lycopene and β−carotene concentrations from spectral data in hexane were consistent with and closely aligned with the findings reported by Fish [12] and Diacon et al. [16]. Similarly, the equations for lycopene and β−carotene determination in FAEE derived from the transesterification of Krabok seed oil exhibited results comparable to those reported by Diacon et al. [16] for FAEE obtained from the transesterification of sunflower oil. Notably, this study represents the first to utilize FAEE derived from Krabok seed oil as a solvent for extracting lycopene and carotene from tomato pomace.

$$A_{503} = 0.223_{L/mg}[lyc]_{mg/L} + 0.0997_{L/mg}[\beta - car]_{mg/L} \quad (7)$$

$$A_{450} = 0.2256_{L/mg}[lyc]_{mg/L} + 0.2232_{L/mg}[\beta - car]_{mg/L} \quad (8)$$

Table 2. Equations for the quantitative determination of lycopene and β−carotene in hexane solvent (mg/L).

Solvent	Lycopene (mg/L)	β−Carotene (mg/L)
Hexane	$[lyc] = 5.662\ A_{503} - 0.896\ A_{450}$	$[\beta-car] = 4.433\ A_{450} - 3.150\ A_{503}$
FAEE	$[lyc] = 8.183\ A_{503} - 3.655\ A_{450}$	$[\beta-car] = 8.176\ A_{450} - 8.272\ A_{503}$

Note: A refers to the absorbance value.

3.3 Quantification of lycopene and β−carotene in tomato extracts

Based on the analysis of lycopene and β-carotene extracted using conventional maceration extraction (ME) and ultrasonic-assisted extraction (UAE) with hexane and FAEE solvents, the hexane-extracted samples were diluted 40-fold, and the FAEE-extracted samples 25-fold before measurement. The different dilution factors were necessary to ensure that absorbance values fell within the linear range of the calibration curves. Because hexane extracts contained higher initial concentrations of lycopene and β-carotene than FAEE extracts, a greater dilution factor (40-fold) was applied, while only a 25-fold dilution was sufficient for FAEE. The absorbance values were then substituted into the equations provided in Table 2 to calculate the lycopene content in mg/L, and the results were subsequently expressed as mg/100 g dry weight (DW), as shown in Figure 4 (a) and (b). Statistical analysis confirmed that these differences were significant ($p < 0.05$), as indicated by the letter annotations in Figure 4. Overall, the extraction efficiency depends on the solvent properties, particularly viscosity, which affects the dissolution of bioactive compounds and the cavitation process in UAE [7, 17]. When comparing the two extraction methods using hexane and FAEE solvents, it was observed that the lycopene content extracted with hexane ranged from 67.97 ± 1.14 to 75.33 ± 4.02 mg/100 g DW, whereas the FAEE solvent yielded a range of 59.92 ± 0.58 to 63.60 ± 1.86 mg/100 g DW. Similarly, the β−carotene content extracted with hexane ranged from 10.512 ± 0.34 to 11.499 ± 0.79 mg/100 g DW, while the FAEE solvent yielded 1.629 ± 0.13 to 7.013 ± 0.68 mg/100 g DW.

To provide clearer evidence of the comparative extraction performance, quantitative differences between maceration extraction (ME) and ultrasonic-assisted extraction (UAE) were evaluated. UAE with hexane improved lycopene yield by approximately 10% and β-carotene yield by 9% relative to ME, while UAE with FAEE increased lycopene yield by about 7%. Notably, the UAE with FAEE enhanced β-carotene yield by nearly 340% compared to ME, highlighting the substantial benefit of sonication in overcoming the limitations of FAEE's higher viscosity. These results quantitatively confirm that UAE is more efficient than ME in recovering both lycopene and β-carotene. These findings suggest that hexane is more efficient in extracting lycopene and β−carotene than FAEE. The higher extraction efficiency of hexane may be attributed to its lower viscosity (0.44 cSt) compared to FAEE (1.73 cSt). The lower viscosity of hexane facilitates better dissolution of bioactive compounds during the maceration extraction process. Furthermore, in ultrasonic−assisted extraction, the higher viscosity of FAEE can hinder the cavitation process, which is crucial for effective extraction. Cavitation requires the sound pressure during the expansion phase to overcome the intermolecular forces of the liquid. As viscosity increases, the intermolecular attraction becomes stronger, making cavitation more difficult. Consequently, the higher viscosity of FAEE reduces its efficiency as a solvent compared to hexane [18–20].

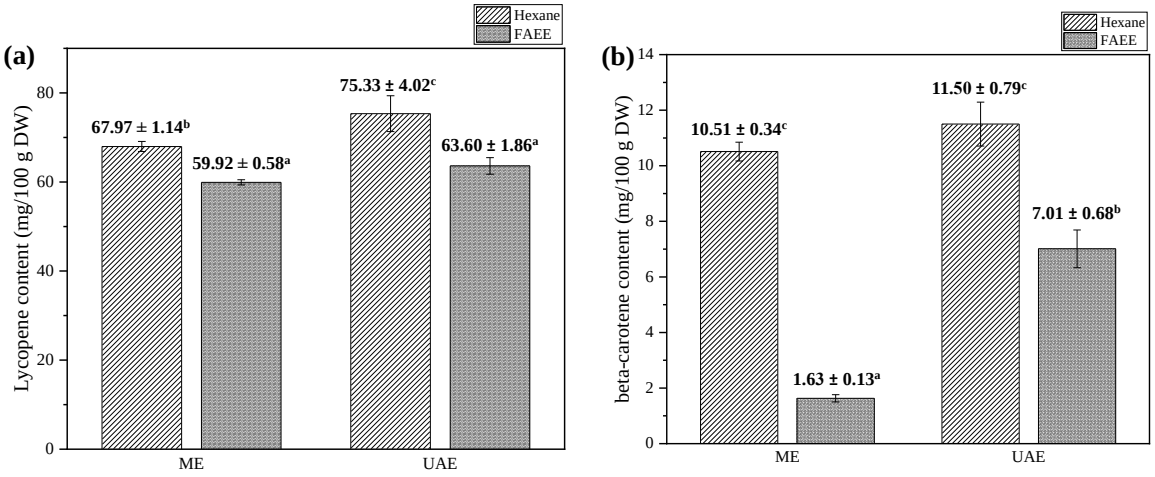

Figure 4. (a) Amount of lycopene extracted from tomato pomace and (b) amount of β−carotene extracted from tomato pomace using conventional maceration extraction (ME) and combined ultrasonic extraction (UAE) with hexane and FAEE solvents. Note: The values represent the mean of three replicates. Different letters within the same column indicate statistically significant differences at the 95% confidence level.

3.4 Antioxidant activity of tomato pomace extracts measured by the DPPH method

The antioxidant activity of tomato pomace extracts was evaluated using DPPH radical inhibition, as described in Section 2.4. In this assay, the DPPH solution without any sample (A_0) was used as the positive control to provide a reference for maximum radical activity and to validate the effectiveness of the extracts in scavenging free radicals. Two extraction methods—conventional maceration extraction (ME) and ultrasonic-assisted extraction (UAE)—were compared using FAEE and hexane as solvents (Figure 5(a)). The results showed that FAEE performed better than hexane in both methods. Specifically, ME with FAEE yielded a DPPH inhibition of 70.29 ± 0.35%, while UAE with FAEE produced 77.16 ± 4.44%, significantly higher than hexane ($p < 0.01$). The distinct polarity of FAEE allows the simultaneous extraction of both non-polar carotenoids and polar phenolic compounds. However, the observed DPPH inhibition did not correlate directly with the measured carotenoid content of lycopene and β-carotene. This discrepancy may arise from the combined antioxidant effect of multiple bioactive compounds, including phenolics, which can act synergistically with carotenoids. Therefore, the higher antioxidant activity observed with FAEE likely reflects the contribution of both carotenoids and phenolic compounds rather than carotenoids alone. The combined antioxidant activity of FAEE* and the extracted compounds was evident, with DPPH inhibition reaching 91.65

± 0.50% (ME) and 92.06 ± 0.89% (UAE), showing a statistically significant synergistic effect compared to FAEE-based extracts alone ($p < 0.05$). Additionally, UAE further improved extraction efficiency by disrupting plant cell walls, facilitating better solvent penetration, and increasing the rate of mass transfer, resulting in higher extraction of bioactive compounds. Beyond the performance, FAEE also offers an environmentally friendly alternative to hexane, as it is less harmful and does not evaporate during extraction, supporting a more sustainable process [21–25].

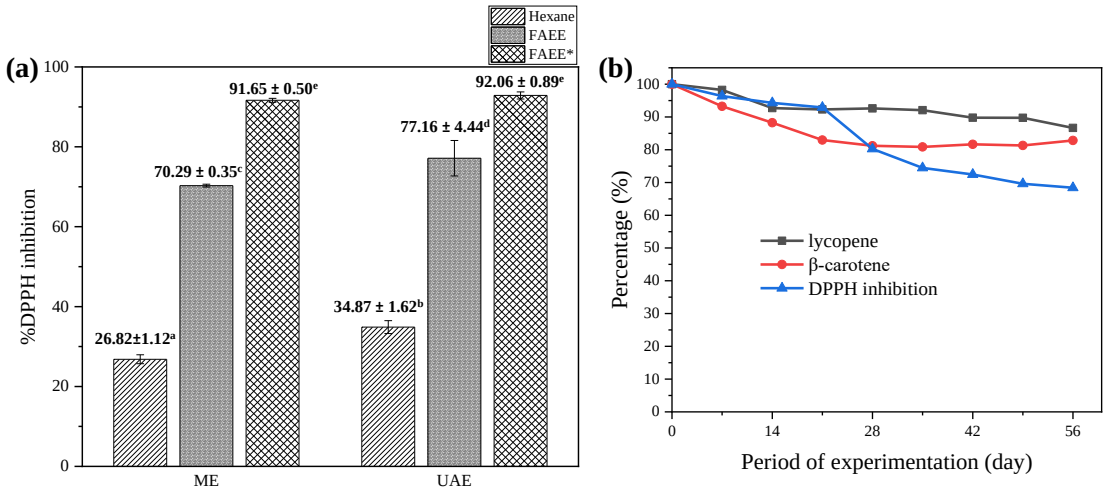

Figure 5. (a) Percentage of DPPH radical inhibition in tomato pomace extracts produced using different techniques. The positive control (DPPH solution without sample, A_0) was used to indicate maximum radical activity, and the calculation method is described in Section 2.4 (Equation 4). (b) A study on the stability of lycopene and β–carotene, along with the percentage inhibition of DPPH radicals, at different time intervals for tomato pomace extracts obtained using the UAE technique with FAEE as the solvent. Note: The values represent the mean of three independent replicates. Different letters within the same column indicate statistically significant differences at the 95% confidence level. *FAEE** Indicates the total antioxidant activity of FAEE.

3.5 A study on the stability of lycopene and β–carotene

The stability of lycopene and β-carotene in tomato pomace extracts obtained through ultrasound-assisted extraction (UAE) using fatty acid ethyl esters (FAEE) as a solvent was assessed over a 56-day period at 4 °C. The results showed that both compounds experienced an initial decline over the first 28 days, after which their levels stabilized. By day 56, lycopene had decreased by approximately 10%, and β-carotene by 15%, as shown in Figure 5(b). This study was conducted as a preliminary investigation with triplicate measurements (n = 3) at each time point. The reported reductions in lycopene (~10%) and β-carotene (~15%) over 56 days are presented as mean ± SD and should therefore be interpreted descriptively. Due to the limited sample size, formal inferential testing (e.g., repeated-measures ANOVA with post hoc comparisons) was not conducted in this work. Such analyses will be applied in future, larger-scale studies to confirm statistical significance and to report p-values and 95% confidence intervals. This indicates that while FAEE, derived from Krabok seed oil, is slightly less efficient than hexane in initial extraction, it offers considerable advantages in preserving the stability of these sensitive compounds. In contrast, hexane-extracted samples degraded rapidly, becoming unquantifiable after just 7 days. The enhanced stability observed in FAEE extracts can be attributed to its slight polarity, which provides stronger solute–solvent interactions than non-polar hexane. These weak dipole interactions and hydrogen bonding can protect carotenoids from pro-oxidant environments, thereby slowing oxidative degradation [16, 21, 22]. Moreover, FAEE derived from Krabok seed oil may contain endogenous bioactive compounds, such as tocopherols and phenolics, which can act synergistically with carotenoids to enhance antioxidant stability. Our previous study confirmed the synthesis and applicability of Krabok-derived FAEE as a functional, slightly polar solvent [11]. These properties likely contributed to the improved preservation of carotenoids compared to hexane.

The study further revealed a gradual decrease in antioxidant activity, with a more pronounced decline observed after the 21st day. Despite this, antioxidant efficiency remained above 70% by the 56th day. This suggests that, in addition to lycopene and β-carotene, other bioactive compounds might have degraded over time, with FAEE effectively mitigating the degradation and maintaining overall stability. These observations suggest that, in addition to lycopene and β-carotene, other minor bioactive compounds such as phenolics, flavonoids, and endogenous antioxidants present in the tomato pomace extracts may contribute significantly to the overall radical scavenging activity. The gradual decline in DPPH inhibition over time, despite relatively small losses in lycopene and β-carotene, suggests that these additional compounds are likely more susceptible to degradation under the applied storage conditions. FAEE, due to its slight polarity and ability to form weak dipole interactions and hydrogen bonds, may help stabilize these labile compounds, thereby mitigating the overall loss of antioxidant activity. Moreover, the protective effect of FAEE could be synergistic, where carotenoids and other bioactive compounds act together to preserve their radical scavenging capacity. This reinforces the importance of solvent selection not only for extraction efficiency but also for the long-term stability of complex mixtures of bioactive compounds, particularly when valorizing food waste for nutraceutical or functional food applications. Future studies incorporating correlation analyses between individual bioactive contents and antioxidant activity would provide quantitative insights into these synergistic effects and further substantiate the role of FAEE in maintaining extract stability.

These findings align with prior research indicating that antioxidants such as lycopene and β-carotene are prone to degradation under suboptimal storage conditions, and that the choice of solvent significantly impacts their stability [11, 16, 21, 22]. In addition, the UAE has been shown to improve extraction efficiency and compound stability by breaking cell walls and enhancing solvent penetration, as corroborated by the studies of Sharma et al. [7], León-López et al. [23], and da Silva Lima et al. [26]. Thus, employing FAEE for the extraction of lycopene and β-carotene from tomato waste offers a sustainable and eco-friendly alternative to conventional solvents. Combined with the UAE, this approach achieves higher extraction yields and better stability of bioactive compounds, supporting the sustainable valorization of tomato waste for applications in natural colorants, nutraceuticals, and functional foods. These results indicate that tomato pomace extracts obtained via FAEE-based UAE not only retain significant antioxidant activity over extended storage periods but also offer a practical and sustainable source of stable bioactive compounds. Such extracts have potential applications in the production of natural colorants, nutraceutical ingredients, and functional food products, providing value-added utilization of tomato waste while supporting environmentally friendly and cost-effective manufacturing processes.

4. Conclusions

In summary, this study demonstrates that fatty acid ethyl esters (FAEE) derived from Krabok seed oil are an effective and environmentally friendly solvent for extracting bioactive compounds, specifically lycopene and β–carotene, from tomato pomace. The ultrasonic–assisted extraction (UAE) method outperformed conventional maceration extraction (ME) in terms of efficiency, and FAEE provided superior antioxidant activity compared to hexane. Stability tests revealed that FAEE–extracted compounds maintained their integrity over a longer period, showing minimal degradation, while hexane–extracted samples deteriorated rapidly. These findings highlight the potential of FAEE as a sustainable and efficient solvent for the extraction of valuable bioactive compounds, contributing to the development of eco–friendly methods for utilizing tomato by–products in the food and cosmetic industries. Future research could focus on optimizing the extraction conditions further and exploring the scalability of this method for industrial applications.

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