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Enzyme-assisted extraction for the recovery of bioactive chemicals from Vietnamese palm fruit peel

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ABSTRACT

Palm fruit peel is regarded as agricultural waste and is inadequately exploited, even though it contains a high concentration of bioactive compounds. This work aimed to enhance the extraction process of vitamin C, total polyphenols, total flavonoids, total carotenoids, and extract recovery efficiency from the fruit peel of the palm (*Borassus flabellifer L.*) using an enzyme-assisted extraction method. The study was designed to optimize the critical parameters of the enzyme-assisted extraction process. The investigation involved two main stages: (i) assessing the influence of enzyme concentration (1-4%) and pH (4.0-5.5), and (ii) analyzing the impact of extraction duration (15-60 min) and temperature (40-55°C). This systematic approach was employed to find the ideal combination of conditions that would maximize the simultaneous recovery of all targeted compounds. The optimal conditions were determined to be a 2% enzyme concentration, a pH of 4.5, a temperature of 50°C, and an extraction duration of 45 minutes. Under these optimal parameters, the study successfully achieved an extraction yield exceeding 90%. Crucially, these conditions also succeeded in maximizing the recovery of all targeted bioactive compounds, including vitamin C, total polyphenols, total flavonoids, and total carotenoids. This work offers significant benefits by not only enhancing the potential value of palm peel by-products but also by providing a strong theoretical foundation for their industrial-scale application. The successful transformation of this agricultural waste into a valuable resource positions palm fruit peel as a promising raw material for high-demand sectors, including the functional food, cosmetic, and nutraceutical industries.

Contribution/Originality: This study presents an improved enzyme-assisted extraction technique for obtaining important bioactive chemicals from palm fruit peel, an underutilized agricultural byproduct. It provides specific, novel parameters (45 min, 50°C, 2% enzyme, pH 4.5) that significantly enhance the yield, offering a theoretical basis for industrial-scale applications and waste valorization.

1. INTRODUCTION

The palm tree (*Borassus flabellifer* L.) is a tropical species prevalent in Southeast Asia, particularly in Vietnam, with significant economic importance due to its various products derived from the fruit, sap, and trunk. The processing of palm fruit frequently results in the disposal of the outer shell, leading to resource wastage and an

increase in agricultural by-products (Dao, Ngan, Duy, & Ngoc, 2025). Recent studies indicate that the peels of various fruits are rich in bioactive compounds, including vitamin C, polyphenols, flavonoids, and carotenoids (Dao et al., 2025; Dixit, Muruganandam, & Moorthy, 2025; Rifna, Misra, & Dwivedi, 2023; Saleh et al., 2021). Vitamin C (ascorbic acid) is a water-soluble antioxidant crucial for collagen formation, bolstering immunity, and neutralizing free radicals (Alberts, Moldoveanu, Niculescu, & Grumezescu, 2025). Polyphenols and flavonoids are secondary plant metabolites exhibiting potent antioxidant properties, safeguarding cells against oxidative stress, promoting cardiovascular health, and possessing anti-cancer effects (Pogorzelska-Nowicka, Hanula, & Pogorzelski, 2024). Carotenoids are lipophilic molecules that exhibit provitamin A activity and safeguard the organism against photonic radiation damage, thereby aiding in the prevention of degenerative disorders (Atencio, Verkempinck, Reineke, Hendrickx, & Van Loey, 2022).

Enzyme-assisted extraction (EAE) is an eco-friendly and effective technique for extracting bio-compounds from plant sources. Pectinase can hydrolyze cell wall polysaccharides, release intracellular chemicals, and enhance recovery efficiency (Gligor et al., 2019; Łubek-Nguyen, Ziemichód, & Olech, 2022; Poblete, Aranda, & Quispe-Fuentes, 2025). Enzyme activity is influenced by pH, temperature, enzyme concentration, and reaction duration. For instance, vitamin C and flavonoids are susceptible to heat and pH variations, but carotenoids are prone to oxidation at elevated temperatures and prolonged durations (Alberts et al., 2025).

Presently, research on the extraction of bioactive chemicals from by-products of tropical fruits, including mango, papaya, and rambutan, has been documented; nevertheless, information regarding palm fruit peel remains scarce. No systematic study has been conducted on the concurrent impacts of factors during extraction on the bioactive components from this material. Therefore, this study aims to assess the effect of enzyme concentration and pH on vitamin C content, total phenolic content (TPC), total flavonoid content (TFC), total carotenoid content, and extraction yield (EY), and also examine the influence of extraction temperature and time under the best enzyme concentration and pH identified in the experiment. The research findings will serve as the scientific foundation for the application of EAE in the extraction of large-scale palm fruit peel by-products. The utilization of bioactive compounds from palm peel not only reduces environmental impacts but also introduces new commercial value to the processing sector.

2. MATERIAL AND METHODS

2.1. Material

Ripe palm fruits were obtained from the Tri Ton area in An Giang, Vietnam. Fruits of uniform size and moderate ripeness were chosen to guarantee uniformity. The fruits were meticulously rinsed with purified water to eliminate any surface contaminants and pollutants. Subsequently, the outer shell of the mature palm fruit was meticulously removed.

The enzyme preparation Pectinex Ultra SP-L (Novozymes, Denmark) was used in this research, with an activity of 2.5 IU/mL, an optimal operating temperature of 40-55°C, and a suitable pH of 4.0-5.5.

2.2. Experiment Procedure

In the initial experiment, the extract was obtained using water as a solvent at varying pH levels (4.0-5.5, adjusted with citric acid), incorporating different ratios of pectinase enzyme (1-4%). After selecting the optimal settings in the initial experiment, these parameters were used as constants for the subsequent experiment. The second experiment involved enzyme extraction conducted for 15-60 minutes at temperatures ranging from 40 to 55°C to facilitate the disintegration of the fibrous matrix, thereby enhancing the efficiency of ripe palm fruit flesh recovery. The experimental parameters were based on the optimal operating conditions of temperature and pH of the pectinase enzyme preparation, as well as on the exploratory tests conducted by the authors. The resulting liquid was filtered through a sieve to remove solid remnants. The filtration stage aimed to eliminate pulp, thus facilitating the subsequent analysis of extract quality.

2.3. Determination of Quality of Extract

The concentration of vitamin C (%) was assessed using the methodology outlined by Sharaa and Mussa (2019). The total phenolic content (mg TAE/100 g dry matter - DM) was quantified using the methodology established by Martial-Didier, Hubert, Parfait, and Kablan (2017). The flavonoid content (mg QE/100 g DM) was determined using the methodology established by Andrade et al. (2021). The carotenoid concentration (mg/100 g dry matter - DM) was evaluated using a spectrophotometric method derived from the research of Alamu, Maziya-Dixon, Menkir, Irondi, and Olaofe (2021).

2.4. Data Analysis

All tests were conducted in triplicate, and the data are presented as means accompanied by standard deviations. The experimental data were analyzed using Statgraphics Centurion XV. I software, employing analysis of variance through LSD value at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Effect of pH and Enzyme Concentration on the Recovery Yield and Quality of Extract

Extraction yield, calculated as the percentage of filtrate obtained compared to the mass of raw material, is an important indicator for evaluating the efficiency of the extraction process. ANOVA showed a highly significant effect of pH and enzyme concentration (Figure 1), but the interaction between them was not statistically significant ($P > 0.05$).

The recovery yield of the extract peaked at pH 4.5 (82.55%), declined marginally at pH 5.0 (81.33%), and then more noticeably at pH 5.5 (77.48%) and pH 4.0 (74.03%). This demonstrates that the pectinase enzyme is most active at pH 4.5, hydrolyzing pectin into oligogalacturonates that are soluble in water, lowering viscosity, and causing liquid to be released from the tissue. The enzyme continued to function fairly well at pH 5.0, resulting in an efficiency that was only marginally less than that at pH 4.5. At pH 5.5, however, the enzyme activity is significantly decreased, the plant tissue is not entirely broken down, and more liquid becomes trapped in the pulp, all of which lower the extraction yield. Conversely, at pH 4.0, although the enzyme is still active, the pectin in the palm peel itself can easily gel at too low a pH. This phenomenon has been recorded in jam production at a pH of approximately 3.5-4.0 (Chen et al., 2021). As a result, at pH 4.0, the extract tends to be viscous and difficult to filter, reducing the recovery efficiency.

A similar pattern was observed, with yield increasing significantly from 76.3% to 82.2% when enzyme levels were increased from 0 to 2%. This was followed by a minor decline at 3% (79.6%) and 4% (77.3%). The pectinase degrades the fiber and pectin in the peel, making the plant tissue more pliable, thus facilitating the release of liquid (Belkheiri et al., 2021; Dixit et al., 2025). At 2% enzyme, the cell wall is sufficiently degraded to allow most of the internal liquid to escape, thus maximizing yield. However, at 3-4% of the enzyme used, although tissue degradation may be more intense, the high hydrolysis products, such as soluble pectin, increase the viscosity of the extract, making filtration difficult and reducing the amount of filtrate obtained (Belkheiri et al., 2021) as a large amount of extract remains in the viscous residue (Chandel et al., 2022).

Since the interaction was not significant, it can be concluded that 2% enzyme at pH 4.5 was the optimal independent condition, which resulted in reaching 83% of the liquid. This result is consistent with the understanding that the pectinase enzyme reduces viscosity and releases liquid from pectin and fiber-rich raw materials (Roman-Benn et al., 2023). Many studies on fruit juicing with pectinase addition have also shown that more juice was obtained than without the enzyme (Saleh et al., 2021). This is a practical implementation because an efficient extraction process requires not only a high active ingredient content but also maximum extraction volume to be easily applied to large-scale production.

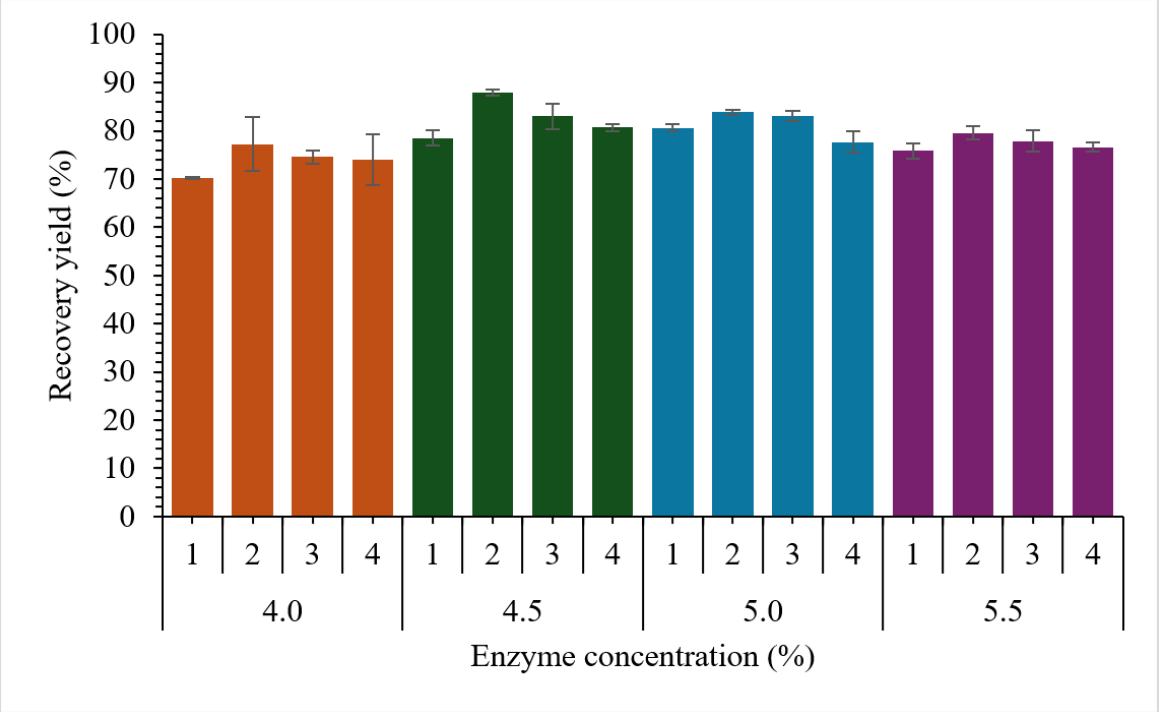

Figure 1. Effect of pH and enzyme concentration on the extraction yield of palm fruit peel.
Note: Error bars represent the standard deviation for each condition.

In terms of bioactive compound content, ANOVA showed that both pH and enzyme concentration had a very significant effect ($p < 0.01$) on the vitamin C content, and there was also an interaction between them ($p < 0.05$) (Table 1). The maximum vitamin C concentration (1.139 mg/g) was found at a pH of 4.5, which is much higher than that at pH 4.0 (0.821 mg/g) and over three times greater than at pH 5.0-5.5 (only 0.334–0.430 mg/g). The reason is that enzyme activity rapidly declines outside the optimal pH range of 4.5, making cell wall hydrolysis less effective at too low or too high pH. The vitamin C content more than doubled when the enzyme concentration was increased from 1% (0.524 mg/g) to 2% (1.063 mg/g); however, the content dropped to 0.593 mg/g and 0.545 mg/g, respectively, when the enzyme concentration was increased to 3-4%. This suggests that moderate enzyme doses (2%) are sufficient to degrade cell walls, releasing vitamin C, while too high doses may increase degradation or create an unfavorable environment for vitamin C (Alberts et al., 2025). The interaction between pH and enzyme concentration was demonstrated by the fact that just 2% enzyme is required to get the maximum vitamin C content at the ideal pH of 4.5, but enzyme efficiency drastically decreases at pHs other than 4.5. For example, using 2% enzyme at pH 5.0 only produces 0.75 mg/g of vitamin C, which is not appreciably more than using 1% enzyme at pH 4.5. Therefore, combining pH 4.5 with 2% enzyme is the optimal condition for pectinase to work effectively, break down the wall structure, and release maximum vitamin C. This result is consistent with the properties of commercial pectinase from *Aspergillus niger*, which usually has optimal activity at around 50°C, pH 4.5 (Mat Jalil, Zakaria, Salikin, & Ibrahim, 2023). In addition, pectinase enzyme in a mildly acidic environment will hydrolyze polysaccharide complexes bound to vitamin C, releasing free vitamin into the extract (Silva, de França, Converti, & Porto, 2019).

Since carotenoids are hydrophobic molecules, water extraction (without enzymes) is not very effective (Butnariu, 2016). The use of enzymes can release carotenoids from the cell membrane and protein-lipid complexes in chromoplasts, helping to increase the amount of carotenoids in the extract (Butnariu, 2016). The results in Table 1 showed that pH and enzyme concentration strongly affected the carotenoid content, with the interaction also being extremely significant. At pH 4.5, the carotenoid concentration was remarkably high, averaging 0.430 mg/g, whereas at pH 5.0 and 5.5, it only reached approximately 0.148-0.150 mg (about 65% lower). The carotenoid content was also much lower at pH 4.0 (0.322 mg/g) than at pH 4.5. In terms of enzyme concentration, 2% is the optimal level, higher than 1%, 3%, and 4%. Ineffective concentrations can be due to excess enzyme causing high viscosity or competitive

interactions, hindering solvent penetration (Dixit et al., 2025); large amounts of released products (galacturonic acid, monosaccharides) can inhibit the enzyme (Silva et al., 2019); and accessory enzymes such as cellulase disrupt cell structure too much, causing rapid release of carotenoids but also degradation due to exposure to oxygen (Gligor et al., 2019). It is evident from the interactions that pH and enzyme concentration are interdependent. Only 1% enzyme produces a significant amount of carotenoids (0.407 mg/g) at the optimal pH of 4.5; 3% enzyme yields a higher amount, and 4% enzyme yields a maximum of approximately 0.456 mg/g. However, increasing the enzyme concentration from 2% to 3% and 4% at pH 5.0–5.5 had a negative effect on carotenoid content.

The polyphenol content was strongly affected by both pH ($p < 0.05$) and enzyme concentration ($p < 0.0001$), with a highly significant two-factor interaction ($p < 0.0001$) (Table 1). Specifically, the greatest polyphenol concentration (0.130 mgTAE/g) was obtained at pH 4.5, which was much higher than at pH 5.0 and 5.5 (0.121-0.123 mgTAE/g). The polyphenol concentration (0.126 mgTAE/g) at pH 4.0 was likewise somewhat lower than that extracted at pH 4.5. This may be attributed to reduced enzyme efficacy at pH 4.0 or the degradation of certain polyphenols in a strongly acidic environment (Cao et al., 2021). Overall, pH 4.5 was optimal for tissue-breaking enzymes, consistent with the pH range of strong pectinase activity (Mat Jalil et al., 2023). Likewise, the maximum polyphenol content (0.146 mg TAE/g) was obtained with 2% enzyme, which was noticeably greater than that of 1% enzyme (0.122 mg TAE/g). The concentrations at 3% and 4%, however, did not increase any further (0.121 mg TAE/g and 0.112 mg TAE/g), which may be due to substrate saturation or an overabundance of enzymes catalyzing side processes related to polyphenol breakdown (Gligor et al., 2019). The pH-enzyme interaction was demonstrated by the pH-dependent optimal enzyme concentration. For example, at a pH of 4.0, using 1% enzyme for extraction achieved the highest polyphenol content (0.156 mgTAE/g); increasing the enzyme to 2% did not improve it (0.150 mgTAE/g). In contrast, at pH 5.5, where enzyme activity is reduced, the 2% concentration yields the highest result (0.161 mgTAE/g). This suggests that at suboptimal pH, increasing the enzyme dose somewhat compensates for the poor activity. The mechanism of extraction involves the enzyme decomposing the polysaccharide network (pectin, cellulose, hemicellulose) in the plant cell wall, thereby releasing phenolic compounds that are bound to the membrane structure by hydrogen bonds and hydrophobic interactions (Łubek-Nguyen et al., 2022). Due to the enzyme, the polyphenols trapped in the cell wall are released more effectively than with pure solvent extraction (Poblete et al., 2025). However, some enzymes can modify the phenolic structure, such as glycosidic chain cleavage, polymerization, or oxidation of polyphenols when used at high doses (Fernandes & Coimbra, 2023). In conclusion, 1-2% enzyme combined with pH 4.0-4.5 and 2-3% enzyme combined with pH 5.0-5.5 are optimal for the highest polyphenol recovery from palm peel, ensuring high enzyme activity and limiting undesirable degradation of polyphenols.

Enzyme supplementation slightly improved the flavonoid content, and the results were significantly influenced by pH ($p < 0.0001$) as well as enzyme concentration ($p < 0.0001$), along with an interaction between the two factors ($p < 0.0001$) (Table 1). The flavonoid output peaked at pH 4.5 (0.277 mgQE/g), which was approximately 1.3 times higher than at pH 5.0–5.5 (ranging from 0.209 to 0.215 mgQE/g) and significantly higher than at pH 4.0 (0.254 mgQE/g). This clearly indicates a sharp decrease in pectinase enzyme activity as the pH approaches neutral, since higher pH levels significantly inactivate the enzyme (Cardoso, Kobltz, Ortiz, Carvalho, & Carvalho, 2019). On the other hand, too low a pH (4.0) is also not ideal because it can partially denature the enzyme or hydrolyze flavonoid glycosides into the less soluble aglycone form (Fujita, Alencar, & Park, 2015). Regarding enzyme concentration, 2% and 3% levels yielded similar results and were slightly higher than 1% or 4% enzyme. Therefore, using enzymes at moderate levels is much more effective than without enzymes, but increasing them too much does not provide additional benefits. The pH-enzyme interaction was demonstrated by the fact that at the optimal pH of 4.5, both 2% and 3% enzyme concentrations produced very high flavonoid content (0.285 and 0.282 mg QE/g) with no significant difference. The mechanism of flavonoid extraction is similar to that of polyphenols, as the enzyme assists in breaking down the pectin/cellulose matrix, releasing flavonoids from their complexes with carbohydrates in the cell wall (Siemińska-Kuczer, Szymańska-Chargot, & Zdunek, 2022).

Table 1. Effect of pH and enzyme concentration on the content of vitamin C, carotenoid, phenolic and flavonoid in the extract.

pH	Enzyme concentration (%)	Vitamin C (mg/g)	Carotenoid (mg/g)	Phenolic (mgTAE/g)	Flavonoid (mgQE/g)
4	1	0.329±0.007	0.356±0.002	0.156±0.001	0.232±0.002
	2	1.301±0.005	0.380±0.007	0.150±0.003	0.257±0.006
	3	0.371±0.023	0.280±0.001	0.107±0.004	0.290±0.001
	4	1.283±0.015	0.272±0.001	0.092±0.003	0.237±0.005
4.5	1	1.056±0.024	0.407±0.002	0.141±0.017	0.257±0.002
	2	1.922±0.047	0.406±0.002	0.132±0.001	0.285±0.004
	3	1.330±0.018	0.452±0.002	0.110±0.002	0.282±0.006
	4	0.247±0.016	0.456±0.002	0.136±0.011	0.283±0.004
5	1	0.342±0.015	0.168±0.013	0.093±0.004	0.240±0.005
	2	0.747±0.015	0.169±0.001	0.139±0.010	0.236±0.002
	3	0.329±0.006	0.133±0.002	0.128±0.001	0.181±0.006
	4	0.301±0.005	0.130±0.004	0.124±0.002	0.181±0.004
5.5	1	0.371±0.023	0.134±0.003	0.098±0.003	0.200±0.005
	2	0.283±0.136	0.168±0.001	0.161±0.002	0.206±0.002
	3	0.342±0.015	0.158±0.021	0.137±0.016	0.227±0.006
	4	0.347±0.015	0.132±0.004	0.094±0.005	0.227±0.006
p-value	pH	0.0244	<0.0001	<0.0001	<0.0001
	Enzyme concentration	0.0006	<0.0001	0.0294	<0.0001
	pH x Enzyme concentration	0.0241	<0.0001	<0.0001	<0.0001

Note: Values shown are mean ± standard deviation and p-value. A p-value less than 0.05 indicates that the difference between values in the same column is statistically significant at the 5% significance level.

3.2. Effect of Extraction Temperature and Time on the Recovery Yield and Quality of Extract

The results indicated that temperature had a remarkable effect, while time had a moderate effect on the extraction efficiency, and the temperature-time interaction was also significant (Figure 2). Both elevated temperature and prolonged time enhanced efficiency by allowing the solvent to penetrate thoroughly and dissolve the dry matter within the material matrix (Gil-Martín et al., 2022). Specifically, the average yield increased from 92.53% at 40°C to 93.55% at 45°C, 96.86% at 50°C, and 96.60% at 55°C. At extraction temperatures of 50°C and 55°C, no significant difference in extraction yield was found, but these values were both significantly higher than those at 40-45°C. Similarly, extending the extraction time from 15 min to 60 min resulted in more liquid, but the increase was not significant (about 2.6% absolute). The 45- and 60-minute extraction intervals produced not substantially different yields (95.71% and 96.54%), and both were greater than the 15-30 minute extraction times (93.38% and 93.9%). The interaction was evident in the fact that at 50-55°C, even 15 min yielded over 95%, while at 40°C it took 60 min to reach approximately 97%. Therefore, increasing temperature had a stronger effect than increasing time on solution recovery. The reason could be that high temperature reduces solvent viscosity and swells the material, so the matter is released from the plant tissue more easily (Mungwari, King'ondu, Sigauke, & Obadele, 2024). Specific results showed that at 60 minutes, 55°C, the highest efficiency is approximately 98%, and the lowest is at 15 minutes, 40°C (~88%). Generally, 45 minutes and 50°C are sufficient to reach about 96–97% efficiency; increasing the temperature and duration slightly (≤2%) only marginally improves efficiency.

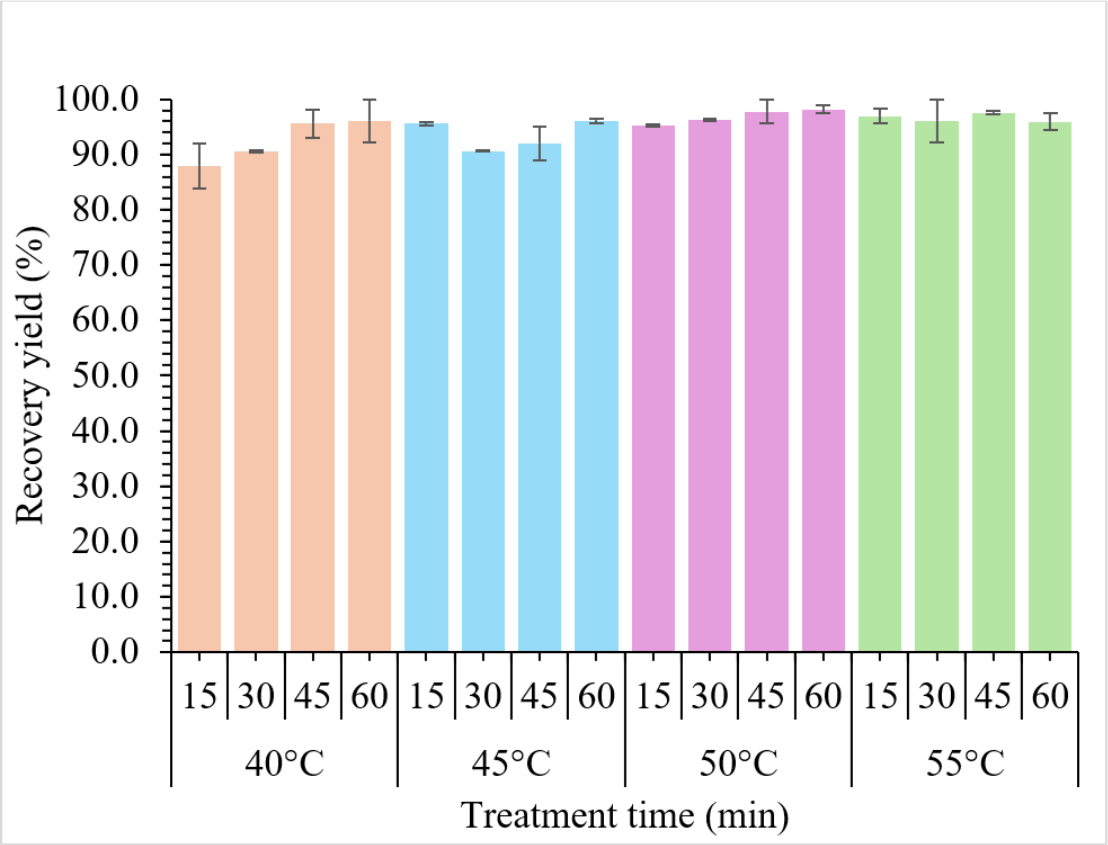

Figure 2. Effect of extraction temperature and time on the extraction yield of palm fruit peel.

Note: Error bars represent the standard deviation for each condition.

ANOVA showed that both extraction temperature and time had significant effects on vitamin C content in the extract, and there was a temperature-time interaction (Table 2). The obtained vitamin C content at 40°C was 1.238 mg/g. It peaked at 50°C (1.355 mg/g) and began to fall at 55°C (1.240 mg/g). The suitable extraction time was also in the middle range. As the duration grew from 15 to 45 minutes, the vitamin C concentration climbed steadily from roughly 1.203 mg/g to a maximum of roughly 1.396 mg/g. At 60 minutes, it slightly fell to roughly 1.248 mg/g. Therefore, the conditions of 50°C and 45 min were suitable for extracting vitamin C from palm fruit peel. This result is reasonable because vitamin C is a water-soluble vitamin that is also sensitive to heat (Jutkus, Li, Taylor, & Mauer, 2015). Therefore, moderate heating time helps to release vitamin C from fruit tissue (Mieszczakowska-Frąc, Celejewska, & Płocharski, 2021) but too long a time or too high a temperature will cause vitamin C to be degraded (Herbig & Renard, 2017). In fact, research has revealed that depending on the temperature and processing time, vitamin C losses can range from 20% to 90% (Mieszczakowska-Frąc et al., 2021).

Carotenoid content in palm peel was quite high and was strongly affected by extraction temperature and time. Both factors and their interaction were statistically significant according to ANOVA analysis (Table 2). Higher carotenoid extraction was the outcome of raising the temperature from 40°C (1.646 mg/g) to 45°C (2.338 mg/g) and 50°C (2.351 mg/g); however, there was no discernible difference between 45°C and 50°C. At 55°C of extraction, the carotenoid content dropped to 2.087 mg/g, significantly lower than the 45-50°C group. These data indicate that the optimal temperature range for carotenoid extraction in the palm peel is 45-50°C, with higher temperatures not providing additional benefits but potentially causing slight damage. This is because carotenoids are relatively more stable than vitamin C but can still degrade at prolonged high temperatures (Atencio et al., 2022). Studies on β-carotene stability have shown that temperatures above 60°C accelerate carotenoid oxidation in the presence of oxygen (Borba et al., 2019; Steenson & Min, 2000). The high temperature also induces cis-trans isomerization of carotenoid (Borba et al., 2019). The 45-minute time period again yielded the highest results when carotenoid content in the

extract increased sharply from 1.887 mg/g (at 15 minutes) to 2.596 mg/g at 45 minutes, then decreased to 2.006 mg/g at 60 minutes. The decrease observed at 60 minutes may be due to carotenoids starting to oxidize or isomerize under the influence of heat and oxygen after a prolonged period (Toydemir et al., 2022). According to the temperature-time interaction, at 45 minutes, very high values (2.603-2.840 mg/g) were obtained at 45, 50, and 55°C. At shorter intervals (15-30 min), however, the high temperature of 55°C was similarly ineffective because there was not enough time for the tissue to be broken down, leading to significantly lower amounts (only 1.598-1.914 mg/g). Similarly, at the long duration (60 minutes) and the temperature of 55°C, the carotenoid content was also only 1.998 mg/g, which is much less than what was extracted for 45 minutes at the same temperature. Therefore, it could be concluded that 50°C and 45 minutes are good conditions to extract carotenoids while avoiding prolonged thermal degradation.

According to Table 2, there was a substantial temperature-time interaction and a significant impact of both extraction temperature and time on the total polyphenol content. Increasing the temperature from 40°C (0.215 mgTAE/g) to 50°C (0.252 mgTAE/g) resulted in more polyphenols being extracted, but when the temperature reached 55°C, the content of polyphenols decreased to 0.218 mgTAE/g. Therefore, the highest content of polyphenols was found in the extract at 50°C and was significantly different from the three remaining temperatures. A similar pattern was observed when the duration was extended from 15 to 45 minutes; the extract's polyphenol concentration rose from 0.205 to 0.249 mg TAE/g before falling to 0.226 mgTAE/g at 60 minutes. The 45-minute period produced the greatest value and differed statistically from the 15- and 30-minute periods. Mild temperature induces the plant cell wall to swell, improving solvent penetration and the efficiency of polyphenol dissolution, which explains why the polyphenol content increases up to 50°C (Pogorzelska-Nowicka et al., 2024; Saleh et al., 2021). However, some phenolic compounds may undergo oxidation or transformation at temperatures over 50°C, which could reduce the observed levels. Furthermore, despite an improvement in diffusion efficiency, prolonged extraction times (60 min) may cause polyphenols to be oxidized by endogenous oxidizing enzymes and ambient oxygen (Gil-Martín et al., 2022; Luangsakul, Kunyanee, Kusumawardani, & Van Ngo, 2025), which would result in a minor drop in content. The temperature-time interaction was demonstrated by the fact that, at 15 minutes, there was no significant difference between 40°C, 45°C, and 50°C, and the polyphenol content ranged from 0.200 to 0.207 mgTAE/g. At longer times, however, the high temperature of 50°C performs better than the low temperature. Several writers also found that extracting fruit or peel at about 50°C and 45-60 minutes produced the maximum polyphenol concentration (Loan, Vinh, & Tai, 2024).

Similar trends were observed for total flavonoid content in the extract under different conditions. Both temperature and extraction time significantly influenced flavonoid content, and the temperature-time interaction also reached statistical significance (Table 2). Specifically, the flavonoid content increased significantly from 15 to 45 minutes (from 0.181 mgQE/g to 0.288 mgQE/g), and then declined to 0.238 mgQE/g at 60 minutes. The best results were obtained after 45 minutes, which also demonstrated a substantial difference from 15 minutes, 30 minutes, and 60 minutes. The impact of extraction temperature was even more evident because, at 40°C, only 0.176 mgQE/g of extract was obtained; at 45°C, this value increased to 0.236 mgQE/g; the maximum value was obtained at 50°C (0.337 mgQE/g); at 55°C, it dropped significantly to 0.198 mgQE/g. Therefore, 50°C for 45 minutes was likewise the ideal extraction temperature for flavonoids. At 55°C, certain flavonoids, particularly flavonoid glycosides, are unstable at high temperatures and can be decomposed or transformed at 55°C (Koop et al., 2022; Lin, Simal-Gandara, Cao, & Xiao, 2023). Additionally, even if the diffusion rate increases at 55°C, evaporation or local boiling may cause the solvent residence time in the matrix to decrease, which would reduce the solubilization efficiency (Efthymiopoulos et al., 2018). The temperature-time connection was also demonstrated by the findings. Forty-five minutes of extraction was enough to produce the most flavonoids before they were broken down at high temperatures of 50 to 55°C. Outperforming the other combinations, the 50°C-45 min sample produced the highest flavonoid concentration (~0.431 mgQE/g), demonstrating the synergy between sufficient time and the right temperature. This conclusion is

consistent with previous studies of plant flavonoid extraction, as the temperature range of 50–60°C is generally optimal for flavonoid recovery due to the balance between release and stability of the compound (Mungwari et al., 2024). According to a survey on microwave-assisted flavonoid extraction, the optimal yield within the measured range was obtained at 50°C and for around 45 minutes (Gao, Min, Fang, & Zhong, 2017).

Table 2. Effect of extraction temperature and time on the content of vitamin C, carotenoid, phenolic, and flavonoid in the extract.

Temperature	Time (Min.)	Vitamin C (mg/g)	Carotenoid (mg/g)	Phenolic (mgTAE/g)	Flavonoid (mgQE/g)
40°C	15	1.153±0.032	1.254±0.049	0.200±0.005	0.147±0.039
	30	1.193±0.065	1.841±0.552	0.206±0.002	0.180±0.058
	45	1.323±0.031	2.111±0.372	0.227±0.006	0.202±0.040
	60	1.280±0.113	1.377±0.374	0.227±0.006	0.175±0.027
45°C	15	1.220±0.108	2.385±0.104	0.207±0.005	0.201±0.066
	30	1.240±0.131	1.806±0.435	0.214±0.004	0.264±0.135
	45	1.360±0.026	2.831±0.045	0.237±0.005	0.281±0.012
	60	1.133±0.015	2.330±0.328	0.232±0.002	0.198±0.042
50°C	15	1.220±0.108	1.996±0.286	0.207±0.002	0.195±0.083
	30	1.230±0.106	2.483±0.093	0.252±0.006	0.307±0.070
	45	1.560±0.098	2.603±0.084	0.282±0.006	0.431±0.010
	60	1.410±0.072	2.320±0.210	0.266±0.004	0.414±0.060
55°C	15	1.220±0.108	1.914±0.561	0.207±0.001	0.181±0.012
	30	1.230±0.106	1.598±0.337	0.236±0.002	0.208±0.034
	45	1.340±0.010	2.840±0.096	0.247±0.053	0.238±0.011
	60	1.170±0.061	1.998±0.355	0.181±0.003	0.164±0.003
p-value	Temperature (Temp)	<0.0001	0.0244	<0.0001	0.0006
	Time (T)	0.0029	0.0006	<0.0001	<0.0001
	Temp x T	0.0472	0.0241	0.0001	0.0261

Note: Values shown are mean ± standard deviation and p-value. A p-value less than 0.05 indicates that the difference between values in the same column is statistically significant at the 5% significance level.

4. CONCLUSION

The results indicated that enzyme-assisted extraction from the fruit peel of *Borassus flabellifer* L period was optimized at a 2% enzyme concentration and pH 4.5. This combination effectively released vitamin C, polyphenols, flavonoids, and carotenoids by hydrolyzing cell wall polysaccharides while preserving the stability of sensitive compounds. Extraction time and temperature also significantly influenced the process, with 45 minutes and 50°C identified as optimal conditions for enhancing compound diffusion into the solvent while minimizing thermal decomposition and oxidation. These optimal conditions resulted in high extract recovery efficiency and significant biological compound content, demonstrating the efficacy of enzyme-assisted extraction technology. This research contributes to the efficient utilization of palm peel and demonstrates innovative and sustainable processing technology for converting agricultural by-products into bioactive raw extracts. This material has potential applications in functional foods, pharmaceuticals, and cosmetics, while also promoting circular economic development in tropical agriculture. The extracts can be added to food products like drinks, dietary supplements, and nutritious snacks as natural antioxidants or fortifiers to increase their nutritional content and prolong their shelf life. The extract may be developed into an active ingredient in herbal medications due to its high level of polyphenols and flavonoids, which are known to have anti-inflammatory, antioxidant, and anti-cancer activities. Because of its anti-aging and skin-protective qualities, vitamin C and carotenoids are highly valued in the cosmetics industry. The extracts provide

a sustainable and natural substitute for synthetic compounds in skincare formulations such as serums, lotions, and sunscreens. Converting palm peel into a high-value resource adds economic value for farmers and reduces the environmental burden of waste disposal. The study, however, concentrated on optimization at the laboratory scale. The economics of using enzymes on a large scale and managing waste must therefore be considered, as well as the process's scalability to an industrial level. Furthermore, the bioavailability and bioefficacy of the extracted compounds in humans should also be tested.

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