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EFFECTS OF ENZYMES ON SAPONINS AND POLYPHENOLS EXTRACTION FROM *Musa balbisiana* PEEL

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ABSTRACT

Musa balbisiana, widely distributed in tropical and subtropical regions, contains fundamental phytochemicals. This study aimed to investigate the effects of cellulase and pectinase enzymes on saponin and polyphenol-rich extractions from *M. balbisiana* peel. The results indicated that enzymatic treatment with cellulase and pectinase at a concentration of 0.6% (v/w), incubated at 40-45 °C for 90 min, yielded the highest saponin content with the total saponin content (TSC) of 17.50 ± 0.62 mg/g_{DM} (cellulase) and 12.20 ± 0.65 mg/g_{DM} (pectinase). Under the same conditions, the highest polyphenol content (TPC) were also obtained, with values of 19.80 ± 0.67 mg_{GAE}/g_{DM} and 13.27 ± 0.58 mg_{GAE}/g_{DM} for cellulase and pectinase treatments, respectively. Under selected enzymatic extraction conditions, the combined use of pectinase and cellulase at a ratio of 1:3, incubated for 90 min at 45 °C, resulted in the highest yields of bioactive compounds with 26.45 ± 0.90 mg/g_{DM} (TSC) and 30.14 ± 0.47 mg_{GAE}/g_{DM} (TPC). The Scanning Electron Microscope (SEM) results also showed that using enzymes changed the cell structure of the starting material and supported the extraction process. The enzyme-assisted extraction method is an effective means of improving the extraction efficiency of saponins and polyphenols from banana peel.

Keywords: Cellulase, *Musa balbisiana* peel, pectinase, polyphenols, saponins.

1. INTRODUCTION

Musa balbisiana is a popular plant in many tropical regions and has many benefits for human health due to its rich supply of biologically active compounds. Someya *et al.* demonstrated the antioxidant capacity of *Musa* sp. peel, attributed to its secondary metabolite levels [1]. *Musa* sp. peels have been studied and found to contain numerous bioactive compounds, including flavonoids, alkaloids, tannins, glycosides, phlobatannins, anthocyanins, and terpenoids, which exhibit strong antibacterial, antidiabetic, antihypertensive, and anti-inflammatory effects [2].

Saponins and polyphenols are common natural compounds found in many precious herbs and are widely used across many fields, especially in pharmaceuticals [3]. There have been many studies on the extraction of saponins and polyphenols using various methods to achieve the best efficiency. Extraction of bioactive compounds using conventional solvent-based methods is often associated with low yields and prolonged extraction times. The final product often contains a small amount of organic solvent, which compromises its quality. Therefore, it is very important to develop an efficient and selective method for extracting bioactive compounds [4]. Nowadays, using green extraction methods such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE) to protect the environment has gained the attention of researchers. These methods help reduce solvent use, energy consumption, waste, and environmental pollution while achieving higher yields. However, MAE and UAE methods focus on breaking the cell wall structure using microwaves and cavitation, which can generate substantial heat over a prolonged period to decompose heat-labile compounds [5]. Meanwhile, EAE relies on the inherent ability of enzymes to catalyse reactions with high specificity and regioselectivity, and to function under mild conditions in aqueous solutions, to disrupt plant cell walls and release saponins and polyphenols [6]. Cell wall components act as a barrier to the extraction of bioactive compounds from plants. Therefore,

the use of certain enzymes to degrade cell wall polymers is an effective approach that has been widely applied across a number of plant species [7].

Banana peels contain major metabolites such as 50% fibre, 7% crude protein, 10% crude fat, 3% starch, essential amino acids (leucine, phenylalanine, threonine and valine), polyunsaturated fatty acids (linoleic acid, α-linoleic acid), micronutrients (iron, calcium, potassium, magnesium, zinc). Banana peels also contain 10 - 20% pectin, 7 - 9% cellulose, 6 - 12% lignin, and 6 - 9% hemicellulose [8]. The addition of cellulase and pectinase enzymes helps break down the components that make up the cell wall, break down the structure, and release the internal components (including water and coloured compounds). In addition, cellulase enzymes catalyze hydrolysis reactions, breaking down the cellulose structure into simple sugars by breaking the 1,4-beta-D-glycosidic bond, making the cell wall loose; the cells are broken down so that water can easily drain, helping to increase the efficiency of extracting biologically active substances [9].

This study evaluated the effects of cellulase and pectinase enzymes on the extraction of saponins and polyphenols from *M. balbisiana* peel. The extraction effectiveness was also evaluated via Scanning Electron Microscope (SEM).

2. MATERIALS AND METHODS

2.1. Materials

The peel was collected from *M. balbisiana* fruits with technical maturity (about 85% ripe), originating from An Hoa ward, Tam Nong district, Dong Thap province, Vietnam. After harvesting, they were transported to the laboratory on the same day, where the raw materials were washed with tap water and impurities removed. They were dried at around 60 ℃ until the moisture content was below 10%. Next, the samples were crushed in a mechanical mill and sieved to obtain a powder of 0.5-1 mm for the entire experiment.

Chemicals: Oleanolic acid (Sigma Aldrich, USA), Gallic acid (Biobasic, Canada), Folin & Ciocalteu's phenol reagent (Sigma Aldrich, USA) and other chemicals with analytical level.

Cellulase enzyme is a commercial product of Viscozyme® L (Sigma, American), in liquid form, brown in colour and with a viscosity of 1.22 g/mL, 10,000 U/g. The pectinase enzyme used is the commercial product Pectinex Ultra SP-L (Novo, Switzerland), 60,000 U/g.

2.2. Methods

2.2.1. Effects of cellulase enzyme on polyphenols and saponins extraction

The mixture of 1 g raw material (dried matter) and distilled water at a ratio of 1/10 (w/v) was added to the cellulase enzyme at the tested concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1% (v/w), pH 5. Then, the mixture was stirred well and extracted in a thermostatic bath (Memmert WNB10, Germany) at 30, 35, 40, 45, and 50 ℃ for 30, 60, 90, 120, and 150 min, respectively. After extraction, it was incubated at 90 ℃ for 5 min to inactivate the enzyme. Then, the mixture was added to 80% methanol until 1/30 (w/v) and incubated at 60 ℃ for 60 min before filtering through Ø110 mm filter paper to obtain a transparent liquid. The extract was adjusted to an appropriate volume to determine the saponin and polyphenol content by UV/VIS spectroscopy at 550 nm and 765 nm [10, 11].

2.2.2. Effects of pectinase enzyme on polyphenols and saponins extraction

The mixture of 1 g raw material (accounting for the dried matter) and distilled water at a ratio of 1/20 (w/v) was added pectinase enzyme with the tested concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1% (v/w), pH 4.5. Then, the mixture was stirred well and extracted in a thermostatic bath (Memmert WNB10, Germany) at 30, 35, 40, 45, and 50 ℃ for 30, 60, 90, 120, and 150 min, respectively. After extraction, it was incubated at 90 ℃ for 5 min to inactivate the enzyme. Then, the mixture was added to 80% methanol until 1/30 (w/v) and incubated at 60 ℃ for 60 min before filtering through Ø110 mm filter paper to obtain a transparent liquid. The extract was adjusted to an appropriate volume to determine the saponin and polyphenol content by UV/VIS spectroscopy at 550 nm and 765 nm [10, 11].

2.2.3. Effects of cellulase and pectinase enzymes on polyphenols and saponins extraction

The mixture, including 1 g of raw material (dried matter) and distilled water at a ratio of 1/20 (w/v), was added to cellulase and pectinase at ratios of 1/1, 1/2, 1/3, 1/4, and 1/5 (v/w), pH 5. The mixture was stirred well and extracted in a thermostatic bath (Memmert WNB10, Germany) at 30, 35, 40, 45, and 50 ℃ for 30, 60, 90, 120, and 150 min, respectively. The mixture was incubated at 90 ℃ for 5 min after extraction to inactivate enzymes. Then, the mixture was added to 80% methanol until 1/30 (w/v) and incubated at 60 ℃ for 60 min before filtering through Ø110 mm filter paper to obtain a clear solution. The extract was adjusted to an appropriate volume to determine the saponin and polyphenol content by UV/VIS spectroscopy at 550 nm and 765 nm [10, 11].

2.2.4. Sample preparation for SEM

Preparation of biological samples for SEM involves several sequential procedures to ensure the preservation of representative structural features. The samples must be processed using appropriate techniques to maintain their original three-dimensional morphology. Additionally, the prepared specimens must be sufficiently stable to withstand the high-vacuum or variable-pressure conditions within the SEM chamber. Typically, the preparation process includes cleaning, trimming or dissecting, chemical fixation, dehydration or drying, mounting, and coating (when required), followed by SEM observation [12].

2.2.5. Analysis methods

Total saponin content (TSC) was determined according to the description of Chen *et al.* [10]. The saponin content was calculated based on the standard curve (the oleanolic acid standard was purchased from Sigma-Aldrich). The mixture, including 2 mL diluted extract, 0.6 mL 5% vanillin solution in acetic acid and 2 mL of 70% perchloric acid, was shaken well and heated in a water bath at 70 °C for 20 min. Then, it was cooled under running water, transferred to a volumetric flask, rinsed, and diluted with ethyl acetate to make 10.0 mL. The saponin content was calculated in mg/g dry matter (mg/g_{DM}).

Total polyphenol content (TPC) was determined colourimetrically using the Folin-Ciocalteu reagent, as described by Singleton *et al.,* with minor modifications [11]. The test consisted of 1 mL of the extract diluted with 5 mL of 10% Folin-Ciocalteu reagent. After 5 min, 4 mL of 7.5% Na_2CO_3 was added, the mixture was shaken well, and the mixture was left at room temperature in the dark for 90 min before measuring absorbance spectrophotometrically at 765 nm. A standard curve for gallic acid (20-100 µg/mL) was prepared similarly, and the results were expressed as mg gallic acid equivalents (mg_{GAE}/g_{DM}).

2.2.6. Statistical analyses

Experiments were repeated 3 times, and the experimental data were presented as Mean ± SD. The ANOVA statistical method was analysed using Minitab 19 software.

3. RESULTS AND DISCUSSION

3.1. Effects of cellulase enzyme on polyphenols and saponins extraction

In enzyme reactions, factors such as the enzyme-to-substrate ratio, solvent type, and reaction conditions (pH, temperature, time, etc.) are important. The effects of cellulase enzyme on saponins and polyphenols extraction are shown in Fig. 1.

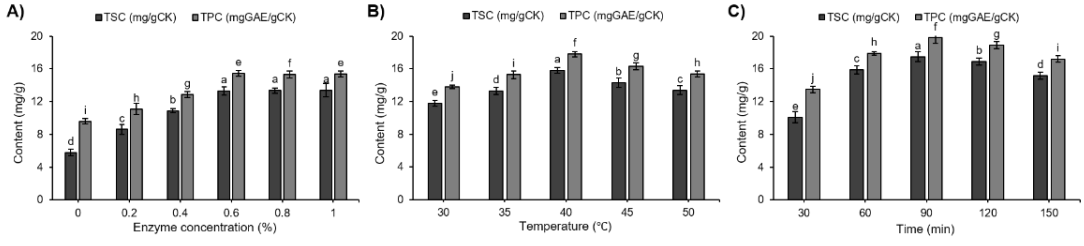


Fig. 1. Effects of cellulase enzyme concentration (A), extraction temperature (B), and extraction time (C) on saponins and polyphenols extraction from *M. balbisiana* peel

In each graph, different letters on the bars represent a statistically significant difference according to ANOVA analysis (α = 0.05)

The enzyme reaction is a reversible reaction influenced by many factors, in which enzyme concentration plays a special role in the enzyme's ability to catalyse substrate decomposition. It can be seen that from 0 to 0.6%, the contents increased from 5.80 ± 0.41 mg/g_{DM} to 13.30 ± 0.49 mg/g_{DM} (saponins) and from 9.60 ± 0.30 mg_{GAE}/g_{DM} to 15.48 ± 0.37 mg_{GAE}/g_{DM} (polyphenols). In enzyme concentrations of 0.8% and 1% (v/w), TSC and TPC were nearly unchanged (Fig. 1A). In principle, with a given amount of substrate, the higher enzyme concentration has a higher efficiency of the enzyme reaction. However, when the enzyme concentration is saturated with substrate, the reaction rate does not increase further as the enzyme concentration increases [13]. On the other hand, more enzymes were added, and more cell wall polysaccharides were hydrolysed, especially those in the polysaccharide-lignin network, releasing additional polyphenol compounds. Another possible mechanism is that the enzyme directly catalyzes the breaking of ether and ester bonds between phenols and plant cell wall polymers [14]. In this experiment, a cellulase concentration of 0.6% (v/w) was selected for further studies.

Each enzyme will work well at an optimal temperature range. The movement of molecules can be affected by temperature, and an optimal temperature can improve enzyme activity [15]. The effects of temperature on saponins and polyphenols extraction were shown in Fig. 1B. The content increased continuously with increasing temperature from 30 °C (TSC: 11.80 ± 0.38 mg/g_{DM}; TPC: 13.80 ± 0.22 mg_{GAE}/g_{DM}) to 40 °C (TSC: 15.80 ± 0.34 mg/g_{DM}; TPC: 17.80 ± 0.34 mg_{GAE}/g_{DM}) and reached the highest at this temperature. The contents of saponins and polyphenols decreased with further increases in temperature; specifically, at 50 °C, they decreased to 13.40 ± 0.52 mg/g_{DM} (TSC) and 15.40 ± 0.37 mg_{GAE}/g_{DM} (TPC). Increasing the reaction temperature can enhance enzyme activity and promote decomposition during the extraction process, but excessive increases or decreases in extraction temperature can inhibit enzyme activity, thereby reducing the value of the obtained extract [16]. The report by Ngo Ke Suong *et al.* (2019) showed that when extracted with the cellulase enzyme at optimal conditions (enzyme concentration of 121 UI/g substrate at 40 °C for 90 min), the protein content obtained was 38.921 mg/g substrate [17]. Therefore, a hydrolysis temperature of 40 °C was used to avoid additional waste in subsequent experiments.

Incubation time also plays a certain role in the enzyme extraction. The appropriate time brings not only extraction efficiency but also significant economic benefits. The results in Fig. 1C showed that at 90 min, the highest saponin and polyphenol content was obtained, reaching 17.50 ± 0.62 mg/g_{DM} and 19.80 ± 0.67 mg_{GAE}/g_{DM}, respectively. As time increased to 150 min, the content of saponins (15.20 ± 0.43 mg/g_{DM}) and polyphenols (17.20 ± 0.38 mg_{GAE}/g_{DM}) in the extract gradually decreased. This showed that the cell wall cleavage activity of the cellulase enzyme is taking place strongly. However, when the diffusion process has reached equilibrium, or in other words, the concentration of the diffusing substance inside the raw material and outside is in equilibrium, there is no difference in the concentration gradient, which leads to the diffusion process slowing down or stopping. The longer the extraction time, the more the extract is exposed to temperature, light, oxygen, and factors that cause oxidation reactions of biologically active compounds and enzyme degradation [18]. This result is similar to that reported by Vu Thuy Anh *et al.*, who found that increasing the enzyme treatment time from 30 min to 90 min increased the total TFC content from 20.39 mg/g_{DM} to 22.32 mg/g_{DM}. However, when the time is increased to 120 min, the total TFC recovery efficiency tends to decrease [19]. Therefore, 90 min was chosen for further studies.

3.2. Effects of pectinase enzyme on polyphenols and saponins extraction

The results in Fig. 2 show the extraction parameters supported by pectinase to achieve the highest saponin and polyphenol content.

According to Fig. 2A, the obtained TPC and TSC content increased continuously from 5.80 ± 0.32 mg_{GAE}/g_{DM} and 4.90 ± 0.41 mg/g_{DM} (0% pectinase enzyme) to 9.10 ± 0.66 mg/g_{DM} and 10.86 ± 0.63 mg_{GAE}/g_{DM} (0.6% pectinase enzyme). This is because the pectinase enzyme cleaves the bond between cellulose and pectin in cells and tissues, releasing soluble substrates into the extract, thereby increasing yield. However, while the TSC continued to increase without significance at 0.8% and 1% enzyme concentration, TPC gradually decreased to 9.45 ± 0.42 mg_{GAE}/g_{DM}. The high enzyme concentration led to greater enzyme hydrolysis and, consequently, better extraction efficiency. However, the excessive enzyme content did not yield higher extraction efficiency or economic benefit [20]. These results were in line with the previous study of Nguyen Hoang Thao Ly *et al.,* which showed that the treatment of

mulberry juice at an enzyme concentration of 0.6% (v/w) for 2 h resulted in the highest TPC of 65.59 mg_{GAE}/g_{DM}, an increase of 30.35 mg_{GAE}/g_{DM} compared to the sample without enzyme [21].

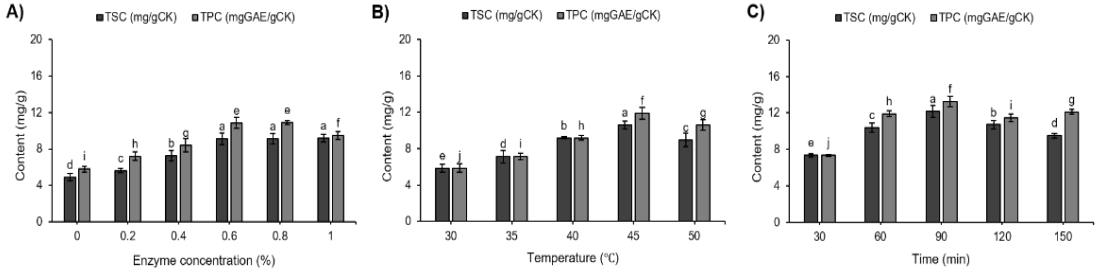


Fig. 2. Effects of pectinase enzyme concentration (A), extraction temperature (B), and extraction time (C) on saponins and polyphenols extraction from *M. balbisiana* peel
In each graph, different letters on the bars represent a statistically significant difference according to ANOVA analysis (α = 0.05)

The data from Fig. 2B showed that the highest TSC and TPC were 10.60 ± 0.43 mg/g_{DM} and 11.90 ± 0.63 mg_{GAE}/g_{DM}, respectively, at 45 ℃. Temperature extraction at 50 ℃ did not give higher saponin and polyphenol content. Increasing temperature has a dual effect on enzyme activity, but the activity decreases when the optimum temperature is exceeded. At the same time, heat treatment increases the solubility and diffusivity of the compounds, reduces the viscosity of the medium, and enhances solvent mass transfer and penetration into the cell, leading to higher extraction efficiency [22]. These findings agreed with those of Pravin *et al.*, who reported that 45 ℃ was chosen to obtain the maximum lycopene from tomato peel powder. The decrease in content at higher temperatures may be due to enzyme denaturation, caused by bond-breaking at its active site [23]. Yashan Li *et al.* also reported that, in the range of 30-45 °C, the anthocyanin content in mulberry fruit extract increased with increasing temperature and reached a maximum of 4.78 mg/g_{DM} at 45 °C [24].

Time is one of the factors affecting the enzyme hydrolysis process; setting the appropriate time through surveys would help improve extraction and economic efficiency. The extraction time was extended from 30 to 90 min, TSC and TPC increased by 12.20 ± 0.65 mg/g_{DM} and 13.27 ± 0.58 mg_{GAE}/g_{DM}, respectively, but the TSC and TPC decreased at extraction time from 120 to 150 min (Fig. 2C). Increasing incubation time resulted in a gradual decrease in the obtained TSC and TPC. This reveals that enzymes degraded cell wall components during the extraction period. At this time, compounds derived from protective chromoplast structures remain exposed to external environmental conditions and may undergo rapid oxidative degradation, leading to a decrease in extract yield [25]. Excessive extraction time leads to oxidation and the formation of unwanted products, reducing the yield of the compounds obtained, wasting energy, and decreasing the equipment's efficiency.

In contrast, short extraction times are not sufficient for enzyme hydrolysis [13]. In agreement with these results, the study of Nguyen Thi Nguyen Thao *et al.* revealed that the highest TPC in mulberry juice was obtained after 90 min of extraction, increasing by 46.8% compared to the control sample. TPC did not increase at longer time (90 - 150 min) [26]. Nguyen Pham Huong Huyen *et al.* also obtained the highest betacyanin content from dragon fruit at 38.88 $mg/100g_{DM}$ in 90 min of extraction [27].

Thus, the optimal conditions for TSC and TPC extraction with pectinase were 0.6% enzyme concentration at 45 ℃ for 90 min.

3.3. Effects of combining cellulase and pectinase enzymes on polyphenols and saponins extraction

In the above experiments, the effects of a cellulase-pectinase mixture on polyphenols and saponins extraction from *M. balbisiana* peel are shown in Fig. 3.

The results in Fig. 3A showed that when using a mixture of cellulase and pectinase at a ratio of 1:3 (v/w), the highest saponins and polyphenols content was 24.12 ± 0.34 mg/g_{DM} and 25.75 ± 0.37 mg_{GAE}/g_{DM}, but when the ratio increased to 1:5 (v/w), the saponins and polyphenols content decreased to 22.96 ± 0.41 mg/g_{DM} and 22.66 ± 0.73 mg_{GAE}/g_{DM}. Cellulase and pectinase enzymes hydrolyse cellulose and pectin into short-chain cellulose and soluble pectin by promoting the release of

intracellular biomolecules and increasing solute concentration in the extract [28]. The higher the enzyme concentration, the stronger the substrate hydrolysis, increasing the release of substrates within the peel. However, when saturation is reached, the efficiency will not increase further [29]. Pham Bao Nguyen *et al.* reported that using cellulase and pectinase enzymes at a ratio of 1:1 significantly increased the recovery efficiency of phenolic compounds (from 53.5 ± 0.4 to 106.7 ± 2.6 mg_{GAE}/g_{DM}) from *Limonia acidissima* fruit [30]. Therefore, a 1:3 cellulase-to-pectinase enzyme ratio was chosen for the following experiments.

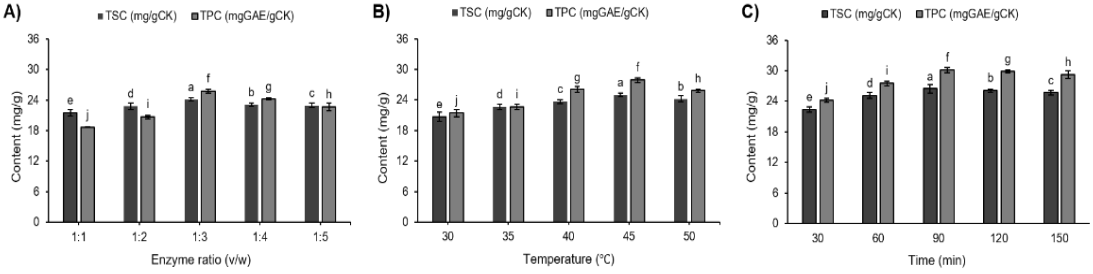


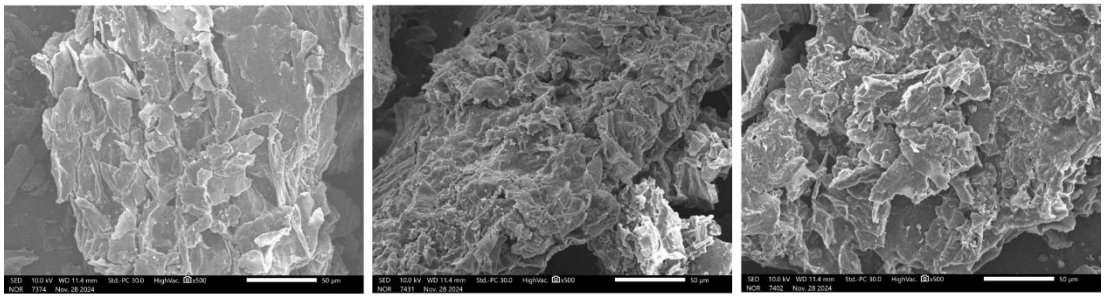
Fig. 3. Effects of cellulase: pectinase enzyme ratio (A), extraction temperature (B), and extraction time (C) on saponins and polyphenols extraction from *M. balbisiana* peel
In each graph, different letters on the bars represent a statistically significant difference according to ANOVA analysis (α = 0.05)

For enzyme-based extraction processes, the optimal temperature must be selected to obtain the highest saponin and polyphenol content. The survey was conducted at temperatures of 30, 35, 40, 45 and 50 ℃. From the results in Fig. 3B, it can be seen that the saponin and polyphenol content tended to increase significantly from 30 ℃ to 45 ℃. At 45 ℃, the saponins and polyphenols content obtained reached the highest values of 24.99 ± 0.33 mg/g_{DM} and 27.88 ± 0.44 mg_{GAE}/g_{DM}, respectively. When the temperature increased to 50 ℃, the saponin and polyphenol content decreased to 24.24 ± 0.59 mg/g_{DM} and 25.80 ± 0.30 mg_{GAE}/g_{DM}, respectively. The report of Tran Thi Thu Tra *et al.* showed that the highest total polyphenol content was obtained from coffee fruit peel and pulp at 50 ℃. Increasing temperature generally reduces the viscosity of the extraction medium, facilitating improved interactions among enzymes, solvents, and target compounds, thereby enhancing the hydrolysis process. However, further increases in temperature may cause enzyme denaturation and the degradation of phenolic compounds, ultimately leading to reduced soluble solids and total phenolic content in the extract [31]. Phan Tai Huan *et al.* also reported that under optimal extraction conditions of 49 °C, pH 4, and enzyme concentration of 0.2% (v/w), the extract from cashew nut shell achieved a total phenolic content of 165.03 mg_{GAE}/g_{DM} [32]. In this study, a temperature of 45 ℃ was selected for the following experiments.

The effects of extraction times (30, 60, 90, 120, 150 min) on the saponins and polyphenols content were shown in Fig. 3C. The results indicated that the saponins and polyphenols content obtained were 22.36 - 25.10 mg/g_{DM} (TSC) and 24.18 - 27.52 mg_{GAE}/g_{DM} (TPC) at 30 min and 60 min extraction, respectively. The saponin and polyphenol contents reached the highest levels of 26.45 ± 0.90 mg/g_{DM} and 30.14 ± 0.47 mg_{GAE}/g_{DM} after 90 min extraction. From 120 min onward, the saponin and polyphenol content tended to decrease. This is consistent with the principle of enzymatic hydrolysis, which requires an appropriate time for the enzyme to act on all organic materials, hydrolyse pectin and cellulose in the plant tissue structure, facilitate the release of intracellular substances, and enhance their diffusion, making the process more effective [33]. Thus, the experimental results showed that the appropriate extraction time was 90 min.

3.4. Evaluation of the structure of banana peel material after extraction

The hydrolysis of cellulase and pectinase enzymes disrupts the structure of *M. balbisiana* peel. These effects were observed in morphology via SEM images (Fig. 4).



(A) (B) (C)

Fig. 4. Morphology of *M. balbisiana* peel after extraction (A), cellulase-assisted extraction (B), and cellulase and pectinase-assisted extraction (C)

The cell wall structure observed under a scanning electron microscope showed that the plant tissue was broken and deformed with signs of shrinking and is gradually separating due to the addition of cellulase enzyme into the sample (Fig. 4B). This is because after performing its catalytic function at the first contact point on the primary wall cellulose, a cellulase molecule can become immobile and ineffective; move to another contact point and continue to catalyze the hydrolysis of the primary wall cellulose; or start catalyzing the hydrolysis of the inner layer or the secondary cellulose layer. In combination with the surfactant, appropriate agitation will reduce enzyme immobilisation, thereby helping them return to solution. The primary wall has shorter cellulose molecules and lower crystallinity than the secondary wall. Therefore, the enzymes penetrate the primary wall structure and hydrolyze it preferentially, causing the cuticle to separate [34]. In Fig. 4C, SEM observations at a magnification of 500× (scale bar: 50 μm) showed that the combined treatment with cellulase and pectinase led to pronounced structural disruption of the cells. The cell walls appeared collapsed and curled, exposing the internal cellular structures (Fig. 4B). This change may be due to the pectinase enzyme hydrolyzing the pectin molecules, breaking the bonds in the molecules, causing the loss of the connection that pectin in its natural form is in the cell wall and can intercalate with other structural polysaccharides and proteins to form insoluble protopectin [35]. In the control shown in Fig. 4A, no structural changes were observed, and the cells remained intact. This result is consistent with the study by Zeng *et al.*, which showed that hydrolysis of corn stover with cellulase for 3 h resulted in a rough surface in the inner part of the rolled cells and clear porous areas. These may correspond to higher glucose conversion, but it was also possible that the internal structures of corn stover were exposed when the grains were ground to a smaller size [36].

4. CONCLUSION

In conclusion, enzyme-assisted extraction significantly improved the recovery of bioactive compounds compared with conventional methods. The suitable conditions for individual enzyme treatments were achieved using 0.6% (v/w) cellulase or pectinase at 40-45 °C for 90 min, resulting in enhanced saponin and polyphenol yields. Moreover, the combined enzyme treatment with a cellulase-to-pectinase ratio of 1:3 (v/v) at 45 °C for 90 min further increased the extraction efficiency, producing the highest concentrations of saponins and polyphenols. Scanning electron microscopy (SEM) analysis confirmed that enzymatic treatment effectively disrupted the cellular structure of the raw material, thereby facilitating the release of bioactive compounds. These findings demonstrate that enzyme-assisted extraction is an effective strategy for improving the recovery of valuable phytochemicals from plant materials.

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TÓM TẮT

NGHIÊN CỨU ẢNH HƯỞNG CỦA ENZYME ĐẾN TRÍCH LY THU NHẬN CHIẾT XUẤT GIÀU SAPONIN VÀ POLYPHENOL TỪ VỎ CHUỐI HỘT *Musa balbisiana*

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Chuối hột (*Musa balbisiana*) được phân bố khắp nơi ở các vùng nhiệt đới và cận nhiệt đới. Nghiên cứu này tiến hành định tính các hoạt chất có trong chiết xuất vỏ chuối hột cũng như khảo sát ảnh hưởng của enzyme cellulase và pectinase đến quá trình trích ly thu nhận chiết xuất giàu saponin và polyphenol từ vỏ chuối hột. Các yếu tố ảnh hưởng đến quá trình trích ly saponin và polyphenol bởi enzyme cellulase với nồng độ enzyme sử dụng tại 0,6% (v/w), nhiệt độ trích ly ở 40-45 ℃ và thời gian 90 phút thu được hàm lượng lần lượt là saponin (17,50 ± 0,62 mg/g_{CK} và 12,20 ± 0,65 mg_{GAE}/g_{CK}) và polyphenol (19,80 ± 0,67 mg/g_{CK} và 13,27 ± 0,58 mg_{GAE}/g_{CK}). Ngoài ra, việc kết hợp hỗn hợp enzyme cellulase và enzyme pectinase thu được hàm lượng saponin và polyphenol cao nhất tại tỉ lệ hỗn hợp hai enzyme là 1:3 với hàm lượng saponin 26,45 ± 0,90 mg/g_{CK} và 30,14 ± 0,47 mg_{GAE}/g_{CK}, nhiệt độ trích ly 45 ℃ và thời gian trích ly là 90 phút. Kết quả chụp SEM cũng cho thấy khi sử dụng enzyme đã làm thay đổi cấu trúc tế bào của nguyên liệu ban đầu và hỗ trợ cho quá trình trích ly. Phương pháp trích ly có sự hỗ trợ của enzyme là một phương pháp hiệu quả, giúp nâng cao hiệu suất trích ly thu nhận chiết xuất saponin và polyphenol từ vỏ chuối hột.

Từ khóa: Cellulase, *Musa balbisiana,* pectinase, polyphenol, saponin, vỏ chuối.