EFFECT OF PECTINASE ENZYME ON EXTRACTION EFFICIENCY AND BIOACTIVE COMPOUNDS OF BLACK MULBERRY JUICE (*MORUS NIGRA* L.) WITH ULTRASOUND-ASSISTED

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ABSTRACT

Black mulberry is widely used in Vietnam because of its flavor and high nutritional value. This study aimed to examine the impact of pectinase enzyme concentration on the extraction recovery efficiency, total polyphenol content (TPC), anthocyanin content (ACN), antioxidant activity (DPPH), and turbidity of black mulberry juice. To process and extract the juice, different concentrations of pectinase (0%, 0.05%, 0.1%, 0.2%, and 0.4%) were used, along with 30kHz ultrasound-assisted conditions. The pectinase enzyme used in this study was obtained by fermenting *Aspergillus niger* with supplemented pectin from red dragon fruits. The results showed that treatment with pectinase enzyme at different concentrations affected the recovery efficiency, TPC, DPPH, ACN, and turbidity. The recovery efficiency reached high values of 88.04% and 88.23% at 0.2 and 0.4% enzyme concentrations, respectively. Similarly, the turbidity of black mulberry juice was also best obtained at enzyme concentrations of 0.2 and 0.4%. However, TPC, ACN and DPPH gave the best results when treated with the enzyme at a concentration of 0.2%.

TÁC ĐỘNG CỦA ENZYME PECTINASE ĐẾN HIỆU SUẤT THU HỒI VÀ HOẠT TÍNH SINH HỌC CỦA NƯỚC DÂU TẰM ĐEN (*MORUS NIGRA* L.) CÓ SỰ HỖ TRỢ CỦA SÓNG SIÊU ÂM

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TỪ KHÓA

Dâu tằm đen
Enzyme pectinase
Sóng siêu âm
Anthocyanin
Polyphenol

TÓM TẮT

Dâu tằm đen được sử dụng rộng rãi ở Việt Nam bởi vì hương vị và giá trị dinh dưỡng cao. Trong nghiên cứu này, enzyme pectinase sử dụng cho quá trình thu nhận dịch dâu tằm đen là enzyme thu được từ quá trình lên men nấm mốc Aspergillus niger trên môi trường có bổ sung pectin thanh long đỏ. Nồng độ enzyme pectinase khảo sát lần lượt là 0; 0,05; 0,1; 0,2 và 0,4% cùng với sự hỗ trợ của sóng siêu âm 30kHz. Dịch chiết dâu tằm đen được thu nhận và đánh giá ảnh hưởng của nồng độ enzyme pectinase đến hiệu suất thu hồi dịch chiết, hàm lượng polyphenol (TPC), hàm lượng anthocyanin (ACN), hoạt tính chống oxy hóa (DPPH), và độ đục của dịch dâu tằm đen. Kết quả cho thấy xử lý enzyme pectinase ở các nồng độ khác nhau ảnh hưởng đến hiệu suất thu hồi, hàm lượng TPC, DPPH, anthocyanin và độ đục. Hiệu suất thu hồi đạt các giá trị cao lần lượt là 88,04% và 88,23% ở nồng độ enzyme 0,2 và 0,4%. Tương tự như vậy, độ đục của dịch dâu tằm đen cũng đạt tốt nhất ở nồng độ enzyme 0,2 và 0,4%. Tuy nhiên, khả năng loại bỏ tổng hợp phenolic, anthocyanin và gốc tự do DPPH mang lại kết quả tốt nhất khi được xử lý bằng enzyme ở nồng độ 0,2%.

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1. Introduction

Mulberry belongs to the Moraceae family. Mulberry is divided into three types: white mulberry (*Morus alba*), black mulberry (*Morus nigra*), and red mulberry (*Morus rubra*) [1]. Compared with the white and red mulberry fruit, the black mulberry fruit is gaining increasing attention due to its attractive color and higher content of phenolic compounds, especially anthocyanin compounds [2]. Mulberry is a widely distributed plant that can thrive in various climates and terrains across the globe. In Vietnam, it is predominantly cultivated in expansive areas along the Red River, Day River, Thai Binh River, and Lam Dong, and scattered in the Mekong Delta region. Mulberry is a perennial woody plant. Its fruit is white or pinkish-white to purple or black, oval or nearly spherical, and about 5 cm in size. The small fruit clusters are arranged along the central axis [3]. Mulberry components, such as anthocyanins, polysaccharides, phenols, alkaloids, and flavonoids, have been extensively studied. Mulberry fruits demonstrate various biological activities, including anticoagulant, antioxidant, anti-obesity, anti-inflammatory, anticancer, and neuroprotective activity [4], [5]. However, mulberry fruits are often fragile, easily damaged, and difficult to transport and preserve. These factors seriously limit the economic viability of mulberry cultivation for fruit production and expansion on an industrial scale.

The pectinase enzyme belongs to the polysaccharide family and is widely used in food and other industries [6]. It is non-toxic and highly effective in hydrolyzing pectin compounds. In the fruit juice process, removing turbidity is a significant concern during fruit juice preparation. Pectin, a sugar-rich polysaccharide found in cereals, vegetables, and fruits, is a major contributor to the turbidity and viscosity of fruit juice. Many studies have demonstrated the effectiveness of using pectinase enzyme in reducing fruit juice turbidity. In 2021, Dal Magro et al. [7] found that the pectinase enzyme enhances press ability and decomposing structure, improving fruit juice yield while ensuring the sensory, stability of the juice, and quality characteristics of the final product. Azimi et al. [8] conducted a study to examine the impact of the pectinase enzyme on juice pretreatment. Their findings revealed that pectinase breaks down the polysaccharide chains and dissolves them in the liquid, effectively reducing. In recent years, pectinase, cellulase, and hemicellulase enzymes have been widely used in fruit juice production to enhance juice recovery, increase total soluble solids, improve clarity, and reduce viscosity and turbidity [9].

The extraction method is the most important factor affecting the efficiency and quality of the fruit juice extract. Instead of traditional crushing and filtering method, we applied the ultrasonic method in the extraction of mulberry juice, which is a modern method that allows the penetration and disruption of cell walls, creating conditions for bioactive substances to escape from the cells and enter the fruit juice [10]. Based on the overall assessment of the benefits of bioactive substances in black mulberry, the topic "Effect of pectinase enzyme on extraction efficiency and bioactive compounds of black mulberry juice (*Morus nigra* L.) with ultrasound-assisted" was conducted by using the pectinase enzyme obtained from *Aspergillus niger* to investigate the recovery efficiency, evaluate the turbidity of the obtained black mulberry juice and the changes in bioactive substances such as total phenolic content (TPC), anthocyanin content (ACN), DPPH free radical scavenging activity, when treated with pectinase enzyme assisted by ultrasound.

2. Methods

2.1. Materials

Black Mulberry (*Morus nigra* L.) was collected from Lam Dong province (**Figure 1a**).

The enzyme used in the study was the Pectinase obtained from *Aspergillus niger* cultured on a medium supplemented with pectin inducer obtained from red-dragon fruit.

2.2. Black mulberry juice collection process

Black mulberry juice was treated with pectinase enzyme at different concentrations: 0%, 0.05%, 0.1%, 0.2%, and 0.4%. This treatment was supported by an ultrasonic bath operating at 35W ultrasonic wave and a frequency of 30 kHz. The temperature was maintained at 40 ºC for 3 hours. Subsequently, the mixture was filtered and centrifuged at 6000 rpm to collect the black mulberry juice **(Figure 1b – f)**. This collected juice was then used to determine turbidity, the total polyphenol content (TPC), anthocyanin content (ACN), and DPPH free radical scavenging ability.

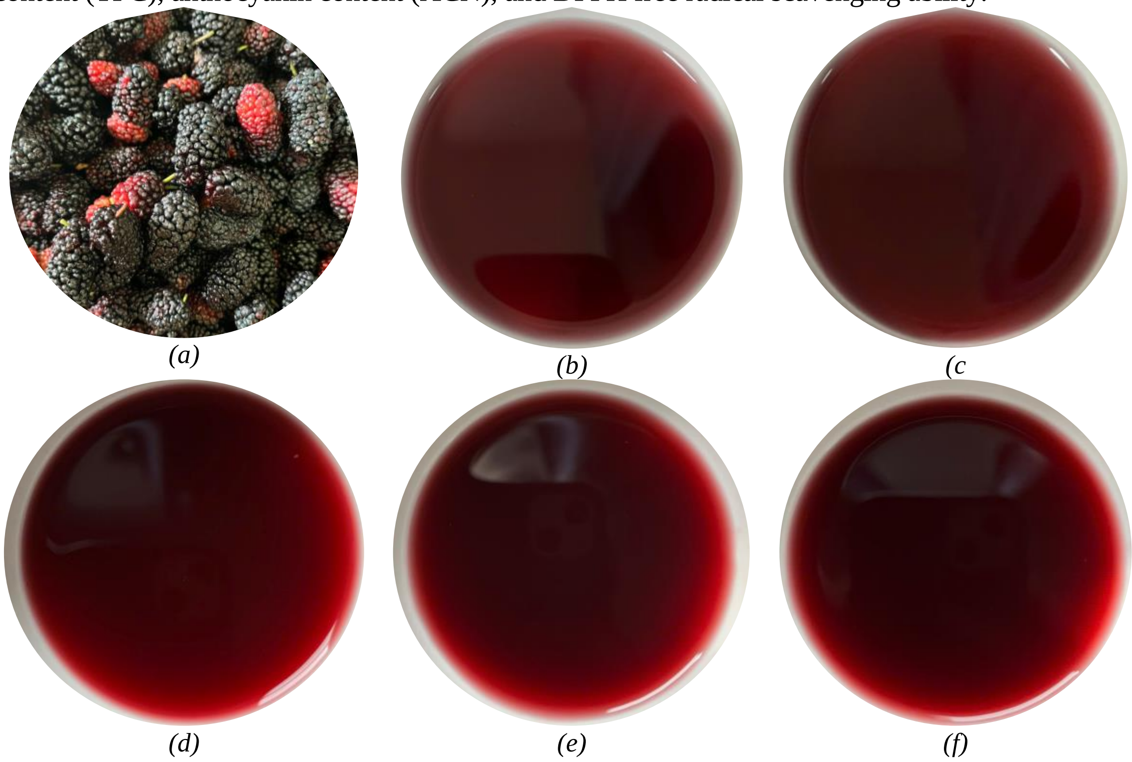

Figure 1. *Research sample: (a) Black mulberry fruits, (b) black mulberry juice treated with 0% enzyme, (c) black mulberry juice treated with 0.05% enzyme, (d) black mulberry juice treated with 0.1% enzyme, (e) black mulberry juice treated with 0.2% enzyme and (f) black mulberry juice treated with 0.4% enzyme*

2.3. Analytical methods

2.3.1. Turbidity (%T)

The turbidity (%T) of the black mulberry juice was determined by the method proposed by Bhat and Goh [11], in which 10 mL of extraction was centrifuged at 6000 rpm at 30 °C for 10 min. Then, the clear supernatant was collected and measured at OD at 660 nm using a spectrophotometer (UV-9000 double beam UV Vis Spectrophotometer, Metash, China), using distilled water as the blank.

2.3.2. Anthocyanin content (ACN)

Anthocyanin content was measured by the pH difference method [12], using 0.025M KCl buffer solution with pH 1.0 and 0.4M $C_2H_3NaO_2$ buffer solution with pH 4.5, the sample absorbance was measured at 520 and 700 nm.

2.3.3. Total polyphenol content (TPC)

The phenolic content was measured according to the Folin-Ciocalteu method reported in ISO 14502-1:2005 [13]. Briefly, 0.6 mL of analytical solution was added to 1.5 mL of diluted FCR and left stand in the absence of light for 3–8 min, followed by adding 1.2 mL of Na_2CO_3 solution (7.5% w/v) and then incubating for further 60 min. The mixture was then determined for absorbance at

765 nm and TPC was presented as equivalent amount of gallic acid in mg per L of extracts (mg GAE/L) based on the gallic acid standard curve.

2.3.4. DPPH free radical scavenging activity

DPPH free radical scavenging activity was performed according to the method described by Brand-Williams et al. with some modifications [14]. Briefly, 2.85 mL of calibrated DPPH reagent was added to 0.15 mL of diluted sample. The reaction mixture was incubated for 30 min in the dark and the absorbance was measured at 515 nm using a UV-Vis spectrophotometer. Eventually, DPPH antioxidant activity was presented as equivalent amount of Trolox in mg per L of extracts (mg TE/L) based on the Trolox standard curve.

2.3.5. Statistical analysis

Statistical analysis of the experimental data was carried out using SPSS Statistics 26.0 (IBM Corp., USA). At a 5% level of significance, Pearson correlation and one-way ANOVA with Tukey's Multiple Range test were conducted to find out whether there were significant differences between the mean values. Triplicates were used for all tests and measurements.

3. Results and discussion

3.1. The recovery efficiency of black mulberry extract

The recovery efficiency of black mulberry juice, when treated with pectinase enzyme at different ratios (0.05, 0.1, 0.2, 0.4% respectively), is shown in Figure 2.

According to the research results, the recovery efficiency of black mulberry juice without using the pectinase enzyme was 70.70%, which is lower than that of black mulberry juice treated with the pectinase enzyme. The recovery efficiency of the black mulberry juice increased proportionally with the ratio of enzymes added to mulberry and reached values of 73.22% (enzyme concentration 0.05%), 82.66% (enzyme concentration 0.1%), 88.02% (enzyme concentration 0.2%), and 88.69% (enzyme concentration 0.4%). The increased recovery efficiency of mulberry juice is attributed to the pectinase enzymes. These enzymes break down pectin, reducing its water-holding capacity. Consequently, water is released into the mixture, enhancing juice recovery efficiency. The rate of pectin hydrolysis reaction is directly related to the concentration of pectinase enzyme. In simpler terms, as the enzyme concentration increases, the rate of hydrolysis increases, resulting in higher juice extraction [15]. The research results showed that it was completely consistent with the study of Nguyen et al. [16] on the effect of enzyme treatment on the quality of mulberry juice. Their results showed that when using pectinase enzyme, the juice recovery efficiency increased by 87.1%, which is significantly higher than when not using enzyme treatment. Similarly, the study by Joshi et al. [17] on the application of pectinase enzyme obtained from apple pomace on the extraction process of fruits such as plum, peach, pear, and apricot also showed that the enzyme increased the juice recovery efficiency from 52% in plum, 38% in peach, 60% in pear and 50% in apricot to 78% (plum), 63% (peach), 72% (pear) and 80% (apricot).

3.2. Turbidity of black mulberry juice

Pectinase enzyme is widely used in food products, especially in fruit juice production technology, not only because of its ability to cut pectin chains to release water, increasing the efficiency of fruit juice recovery, but also because of its ability to clarify fruit juice. The results of the study on the ability to clarify mulberry fruit juice based on the turbidity of the fruit juice are shown in Figure 3.

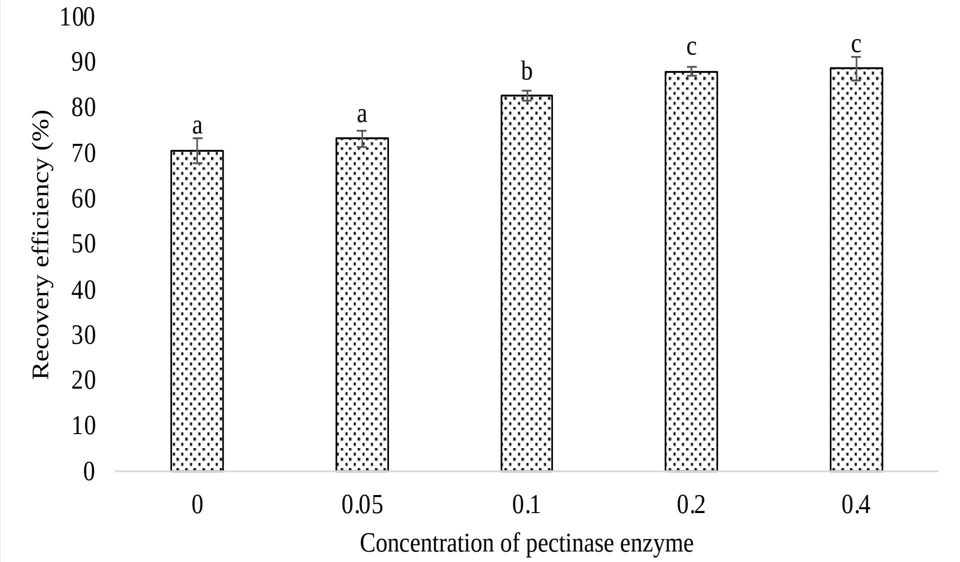

Figure 2. *The recovery efficiency of black mulberry juice when treated with pectinase enzyme concentrations was 0, 0.05, 0.1, 0.2, and 0.4%, respectively*

In some studies, pectinase enzymes have been found to break down pectin bonds in cell walls, which reduces turbidity and settles faster. In general, the research data shows that when increasing the ratio of pectinase enzymes for the juice treatment process, the turbidity of the juice also decreases gradually. For the enzyme concentrations of 0.2% and 0.4%, there is no significant difference (turbidity of 24% and 23% respectively), and the ability to clarify the juice is much better than the juice without enzyme treatment (very high turbidity of 46%). Similar to the study by Lachowicz et al. [18] on the effects of different types of pectinase enzymes on the biological activity, antioxidant capacity, color, and turbidity of chokeberry juice, it was also shown that pectinase enzymes aid in the hydrolysis of pectin, leading to the formation of poly-D-galacturonic acid fragments, which reduces the turbidity in the juice and increases the efficiency of the filtration process.

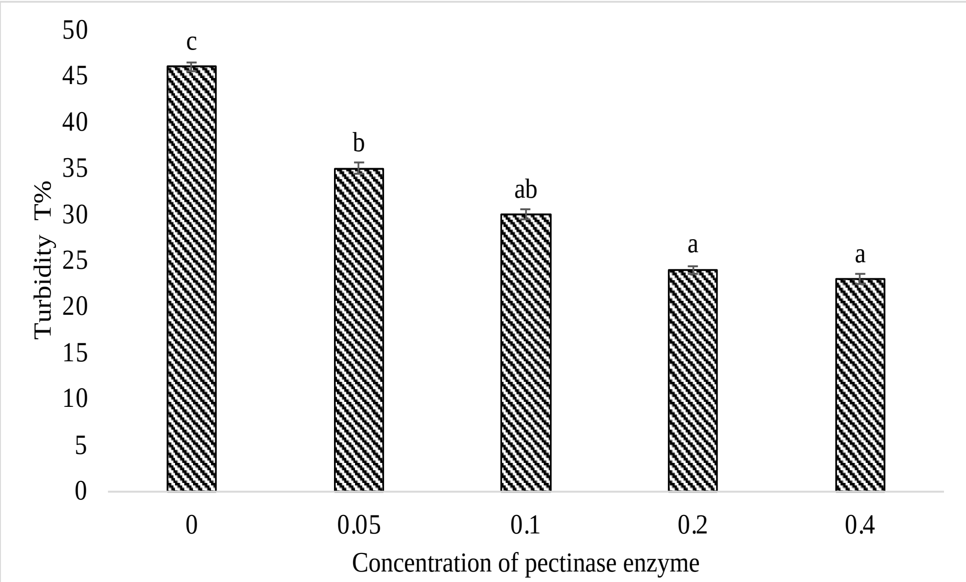

Figure 3. *Changes in turbidity of black mulberry juice when treated with pectinase enzyme concentrations of 0, 0.05, 0.1, 0.2, and 0.4%, respectively*

3.3. Total polyphenol content and DPPH free radical scavenging activity

This research aims to develop products from mulberry juice that can bring health benefits to consumers based on its strong antioxidant capacity. The results of evaluating the influence of pectinase enzyme at different concentrations (0, 0.05, 0.1, 0.2, and 0.4%) combined with ultrasound waves on the bioactive substances of mulberry, including total phenolic content (TPC), and DPPH-free radical scavenging ability, are shown in Figure 4.

The presence of phenolic compounds in plants, including fruits, vegetables, and legumes, is primarily associated with various health benefits such as preventing heart disease, slowing down aging, reducing inflammation, lowering cancer risk, and supporting the prevention of diabetes. The results showed that the total phenolic content (TPC) increased when treated with pectinase enzyme under ultrasound assistance. Specifically, the TPC of black mulberry reached 918.44 mg GAE/L when treated with 0.1% pectinase enzyme. However, at enzyme concentrations of 0.2% and 0.4%, there was a slight decrease in total phenolic content, measuring 899.08 mg GAE/L and 889.76 mg GAE/L, respectively. Nonetheless, these values were still significantly different from the phenolic content in the original sample without enzyme treatment. The decrease in polyphenol content can be attributed to the degradation or transformation of phenolic compounds during the treatment with pectinase enzyme and ultrasound, resulting in a reduction in the measured phenolic content. Furthermore, previous studies have shown that pectinase enzyme can alter the structure of phenolic compounds, causing them to break down or interact with other substances, ultimately decreasing the total phenolic content. These findings align with the research conducted by Ninga et al. [19] on the impact of pectinase enzyme treatment on polyphenol and protein content in guava juice. Their study also demonstrated a decrease in polyphenol content during enzymatic treatment, which was attributed to oxidation to o-quinone. The formation of o-quinone can lead to condensation reactions and complex formation with amino acids and proteins, ultimately resulting in the formation of melanin. The decrease in polyphenol content may also be due to the failure of polyphenols to react and form complexes with other molecules, such as proteins and other polyphenols.

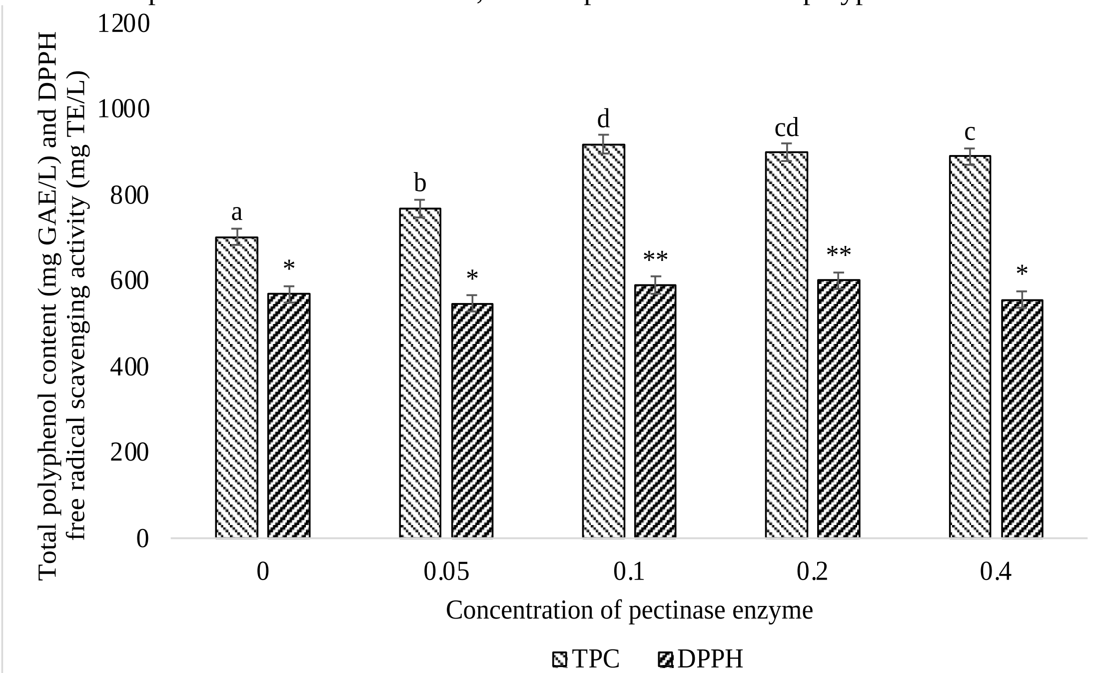

Figure 4. *Changes in total polyphenol content and DPPH free radical scavenging activity were observed when treated with pectinase enzyme at concentrations of 0, 0.05, 0.1, 0.2, and 0.4%, respectively*

DPPH free radical scavenging activity of black mulberry juice extracted by enzyme treatment with different concentrations assisted by ultrasound showed that the black mulberry juice sample treated with 0.2% enzyme concentration gave the best result of 600.87 mg/L, higher than the original sample without enzyme treatment (68.91 mg TE/L) and the sample treated with 0.4% enzyme concentration (555.74 mg TE/L). Some studies by Gani et al. [20] when recovering fruit juice with pectinase enzyme or the study by Khandare et al. [21] also showed that increasing the enzyme concentration to the optimal level will help release phenolic compounds, and flavonoids, increasing antioxidant activity, however, increasing the enzyme concentration too much will affect and reduce the antioxidant activity of fruit and vegetable extracts.

3.4. Anthocyanin content (ACN)

Anthocyanin is a natural plant pigment that is safe and non-toxic to human health and plays an important role in scavenging free radicals in vivo, conveying protections that are anti-cancer, anti-inflammatory, and preventing chronic diseases and diabetes [22]. The results of the study on anthocyanin content in mulberry are shown in Figure 5.

In general, the anthocyanin content increased gradually with increasing pectinase enzyme concentration during ultrasonic treatment. The initial anthocyanin content was 798.12 mg/L and reached the highest value of 988.23 mg/L at 0.4% enzyme concentration, with no significant difference compared to 0.2% enzyme concentration. Similar to the study by Landbo and Meyer [23] on the enzyme treatment of black grapes, it was also shown that enzyme treatment significantly increased the juice yield and increased the total anthocyanin content in black grape juice. This study by Karataş et al. [24] on the effect of commercial pectinase enzyme on anthocyanin content also showed that at 0.2% concentration, it increased the anthocyanin content in black carrot juice samples compared to the original sample without enzyme treatment.

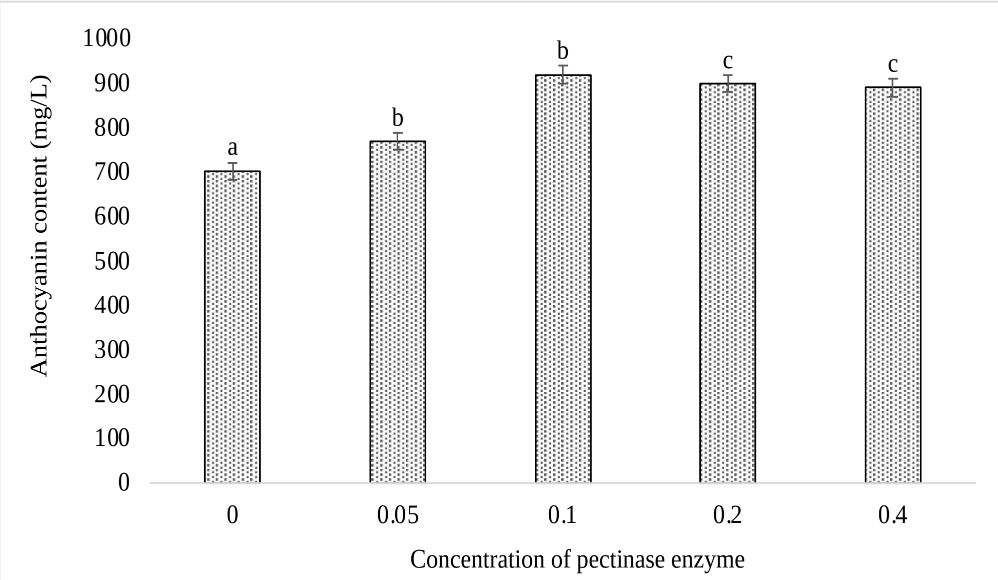

Figure 5. *Changes in anthocyanin content were observed when treated with pectinase enzyme at concentrations of 0, 0.05, 0.1, 0.2, and 0.4%, respectively*

4. Conclusions

This study found that when treating black mulberry with 0.2% pectinase enzyme combined with the support of 30kHz frequency ultrasound waves, the extraction efficiency was improved. When treated with enzyme and ultrasound, the juice recovery efficiency reached 88.04%, higher than when not using enzyme and ultrasound (initially the efficiency reached 70.12%). Along with that, thanks to the ability to break pectin bonds in plants, it reduces the viscosity and sedimentation of mulberry juice, reducing the turbidity of mulberry juice from 46% (initial) to 24%. In addition, the use of enzymes also significantly increased the anthocyanin content (987.04 mg/L), total phenolic content (899.08 mg GAE/L), and DPPH free radical scavenging ability (600.87 mg TE/L).

This study is a premise for further research on the factors and conditions of enzyme and ultrasonic treatment on the biological activity of compounds in mulberry, as well as a premise for research on substances other than enzymes that can process and clarify fruit juice and increase the efficiency of fruit juice recovery for comparison.

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