DOI: https://doi.org/10.62985/j.huit_ojs.vol25.noS2_ICA.234

ANTIOXIDANT ACTIVITY OF PHYCOBILIPROTEIN HYDROLYSATES FROM *Chaetomorpha aerea* USING PROTAMEX ENZYME

Hoang Thi Ngoc Nhon*, Ho Xuan Quynh, Phan Quynh Doan, Ho Thanh Thuong

Ho Chi Minh City University of Industry and Trade

*Email: *nhonhtn@huit.edu.vn*

Received: 11 June 2024; Revised: 16 July 2024; Accepted: 19 July 2024

ABSTRACT

Allophycocyanin (APC), C-phycocyanin (C-PC), and R-phycoerythrin (R-PE) are phycobiliproteins that have garnered attention for their wide range of biotechnological applications in food technology, cosmetics, analytical processes and are extensively used as fluorescent probes. They are the pigment present mainly in red algae and green algae. In this study, we focus on factors affecting the hydrolysis of APC, C-PC and R-PE from *Chaetomorpha aerea* using protamex enzyme to obtain bioactive peptides. Influential factors were investigated, such as enzyme concentration, temperature and hydrolysis time. At an enzyme/substrate ratio of 1.25% v/w, at 50 °C for 90 minutes, the DPPH radical scavenging capacity with the degree of hydrolysis for APC, C-PC and R-PE is (71,25%; 44.80%); (72.67%; 48.36%) and (70.56%; 52.63%).

Keywords: Allophycocyanin, *Chaetomorpha aerea,* C-phycocyanin, R-phycoerythrin, protamex enzyme.

1. INTRODUCTION

Allophycocyanin (APC), C-Phycocyanin (C-PC) and R-Phycoerythrin (R-PE), belonging to the phycobiliprotein family, are the fluorescent pigment proteins with light blue, dark blue and pinkish-red, respectively [1]. APC, C-PC, and R-PE are often abundant in different types of cyanobacteria, such as *Spirulina plantesis, Spirulina maxima, Rhodophytes,* etc., with two subunits α, β and molecular masses of 105 kDa, 30 – 40 kDa and 240 kDa, respectively. They are widely used in biochemical techniques as fluorescent protein probes, especially for flow cytometry, which has become a popular research topic in the food and cosmetic industry [2]. Many studies have been carried out on the extraction and isolation of biomolecules from *S. platensis.* According to Tong X. *et al* (2020), the hydrolysis of *S. platensis* protein by enzymes produces bio-peptides that have anti-diabetic, anticancer, and antibacterial effects [3]. The study of Afify A. *et al* indicated that post-hydrolysed fluid protein from the green algae *Scenedesmus obliquus* also showed that it has high antioxidant activity [4].

Protein hydrolysis is the cleavage of peptide bonds (-CO-NH-) that bind amino acids in a protein molecule in the presence of water. Basically, bioactive protein hydrolysates can be produced from protein sources by various processes, such as chemical hydrolysis, enzymatic hydrolysis by proteolytic enzymes, or microbial fermentation with proteolytic bacteria [5]. Peptides with a molecular mass of less than 6 kDa that hydrolysed from protein have bioactive

properties due to their short structure, composed of 2 to 20 amino acids connected by peptid bonds. For enzymatic hydrolysis, biological properties catalyze the reaction of protein molecules into smaller substances such as short-chain peptides, which have a smaller molecular weight or single-molecule amino acids that can be easily absorbed by the body [6, 7]. Many reported that plant protein hydrolysis is better than animal protein hydrolysis because peptides in plants have many biological functions, including antioxidants, anticancer cells, and anticoagulation [5]. The study by Taghizadeh M. *et al* (2021) about new bioactive peptides *Achillea eriophora* indicated anticancer and antioxidant activities [8]. In addition, research author Ding J. *et al* (2020) studied the protein hydrolysis process in beans and determined good antioxidant activity [9].

C. aerea belongs to the green algae and is a plant widely known among people living near rivers. Fast-growing and developing algae are used to make aquatic feed, fertilizer. In addition, previous studies show that algae *C. aerea* contains a high protein content of about 11 - 23% w/w dry matter and contains many biologically active substances. This shows that *C. aerea* algae has potential value to exploit, thereby opening up many potential research directions on *C. aerea* algae [10]. *C. aerea* is distinguished from morphological characteristics, such as frond dimension, cell size and shape, their mean length/width ratios (LWR), and cell wall constriction, and considered a separate species by the study of Huang B. *et al.* (2016) [11].

To derive biopeptides from *C. aerea*, APC, C-PC, and R-PE were hydrolyzed. The study further investigated the potential applications of APC, C-PC, and R-PE hydrolysates in the realms of food science and pharmaceuticals.

2. MATERIALS AND METHODS

2.1. Materials

C. aerea algae was harvested from a large shrimp farm located in Vinh Chau town, Soc Trang province. For one to two days, the algae was transferred to the laboratory in foam cartons. The algae is thoroughly cleaned at the lab to get rid of contaminants like trash and snails. Afterwards, it was dried in an oven at 60 °C until the moisture content dropped 10%, and then finely ground using a stainless steel grinder. Throughout the course of the investigation, the algae powder was kept in a zip-lock bag and kept in the freezer at -5 °C.

Chemicals: Protamex enzyme is provided by Novozyme (protamex has an activity of 1.5AU-N/g, operates around pH 5.5-6.5, 50-65 ^{o}C). Sodium Hydroxide 98% (NaOH) and Sodium Carbonate (Na_2CO_3) originated from China Xilong. Other analytical chemicals that meet the technical requirements of the laboratory are purchased at Doan Le Chemical Company.

2.2. Methods

2.2.1. Preparing freeze-dried sample

The powder was extracted using 0.05 M phosphate buffer at pH 7.0, with a material-to-solvent ratio of 1:10 (w/v). The extraction was supported by microwave irradiation for 120 seconds at a power of 62 W. Following extraction, the sample was centrifuged at 5,000 rpm for 20 minutes to collect the supernatant. The supernatant was then precipitated with ammonium sulfate at a concentration of 60%. After precipitation, the precipitate was dialyzed using a 14 kDa cellophane membrane. The dialyzed sample was concentrated and loaded onto a Q-Sepharose ion exchange column and eluted with 0.05 M phosphate buffer at pH 5 to

remove impurities. The APC, C-PC, and R-PE were sequentially extracted at different stages. Subsequently, it was eluted with 0.5 M phosphate buffer at pH 7.0. The APC, C-PC, and R-PE were sequentially extracted at different stages. Before measuring parameters, the freeze-dried samples were re-dissolved in a solvent at a ratio of 1:100 (w/v) [12, 13].

2.2.2. Effects parameters on APC, C-PC, R-PE hydrolysis

0.5 g of freeze-dried protein samples of APC, C-PC, and R-PE were mixed with 50 mL of phosphate buffer pH = 6. Then, the mixture was kept in a thermostatic tank with investigated conditions, including protamex enzyme with different concentrations (0.5; 0.75; 1; 1.25; 1.5 %v/w), temperature (40, 50, 60, 70, 80 °C) and time (30, 60, 90, 120, 150 minutes). The samples were inactivated at a temperature of 87 °C for 5 minutes. At the end, the sample was centrifuged at 5000 rpm for 20 minutes and filtered to obtain protein hydrolysate. The obtained filtrate was taken to determine the degree of hydrolysis (DH) and antioxidant activity.

2.2.3. Determination of antioxidant activity of APC, C-PC and R-PE hydrolysate using DPPH

The free radical scavenging capacity of the hydrolysate was determined based on the method of Ganesan K. *et al* (2015); 500 µL of 0.2 mM DPPH solution in methanol was mixed with 500 µL of hydrolysate. The mixture was incubated in a dark place at room temperature for 30 minutes and measured at 517 nm. The control sample did the same but replaced the hydrolysis solution with distilled water. The DPPH radical scavenging capacity of the hydrolysate is calculated according to the following equation [14].

$$\%\ \text{DPPH SCV}\ (\%) = \frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}} \times 100$$

In which:

$A_{Control}$: Absorbance value of the negative control.

A_{sample}: Absorbance value of the test sample.

2.3. Data analysis and processing methods

2.3.1. Analysis methods

2.3.1.1. Protein quantification by Lowry method

The protein content was determined using the Folin-Ciocalteau reagent method as described by Deepachandi B. *et al* (2020).

Required Chemicals:

Solution A: 2 g of Na_2CO_3 (2%) in 50 mL of 0.1 N NaOH

Solution B: 0.5 g of $CuSO_4 \cdot 5H_2O$ in 50 mL of 1% sodium citrate solution.

Solution C: Mixed solutions A and B in a ratio of 49:1 (v/v).

Folin reagent: Diluted two times to an acidity of 1 N

Bovine serum albumin: 0.5 g/mL

Procedure:

Accurately 0.5 mL of the protein solution into a test tube, added 2 mL of solution C, mixed well, and let stand for 10 minutes before adding 0.25 mL of diluted Folin's reagent (diluted twice) to the mixture in the test tube, shook well, and kept for 30 minutes until the

yellow colour of the mixture turns sky blue and reaches maximum colour intensity. The absorbance of the mixture was measured on a spectrophotometer at a wavelength of 750 nm, and determined the absorbance value of the test solution. The procedure was performed with three replicates to take the average value [15].

Control sample: 0.5 mL of buffer to a test tube, add 2 mL of solution C, and proceed with the above steps of the experimental sample.

Calculation formula:

From the standard curve equation and the absorbance of the sample under study, the protein content of sample X (μg/mL) in volume V (L) of the raw material can be determined.

$$\text{Protein content (mg/L)} = \frac{X}{1000 \times V}$$

2.3.1.2. Formol titration method

The content of amino nitrogen was determined by the formol titration method as described by Warakaulle S. *et al* (2024) [16]. Briefly, 5 mL of the protein solution was mixed with 20 mL of distilled water and titrated to pH 8.2 with 0.5 M NaOH. After adding 10 mL of 37% formaldehyde solution, the mixture was titrated to pH 9.2 with 0.05 M NaOH. The volume of base consumed from pH 8.2 to 9.2 was recorded. The amino nitrogen content (X, g/L) was calculated using the formula:

$$X\ (g/L) = \frac{0.0028 \times V \times f}{V_m} \times 1000$$

Where:

0.0028: Amount of N corresponding to 1 mL of 0.2 N NaOH

V: Volume of 0.2 N NaOH consumed

2.3.1.3. Determination of degree hydrolysis

Degree hydrolysis (DH) was defined as the ratio of peptide bonds cleaved in the protein hydrolysate, calculated from the ratio of amino nitrogen (AN) to total nitrogen (TN) [17].

The degree hydrolysis (DH%) of the protein hydrolysate was calculated by the following formula:

$$DH\ (\%) = \frac{\text{Nito formaldehyde}}{N_{total}} \times 100$$

Where:

$N\alpha_{amin}$: Content of amino nitrogen (g/L)

N_{total} Total nitrogen content (g/L)

2.3.2. Data processing methods

Experiments were repeated three times, and data were presented as mean ± SD. Use MiniTab 19 software to statistically analyse experimental data and evaluate differences between samples by ANOVA processing ($p<0.05$). The chart was drawn using Microsoft Excel 2019 software.

3. RESULTS AND DISCUSSION

3.1. Effects of hydrolysis conditions of APC by protamex enzyme

Protamex enzyme is a biological catalyst that helps speed up chemical reaction rates. This enzyme is not only important for all stages of biochemical reactions but also catalyzes reactions inside and outside the cell. During enzymatic hydrolysis, the enzyme-catalyzed reactions are affected due to the denaturation of protein and enzyme molecules. This study was conducted to investigate the effects of APC hydrolysis levels by the protamex enzyme from *C. aerea.* Influential factors included enzyme/substrate ratios 0.5; 0.75; 1; 1.25; 1.5 (%v/w) compared to lyophilized protein mass, respectively. The survey temperature was 40, 50, 60, 70, and 80 (°C), and the survey processing time was 30, 60, 90, 120, and 180 (minutes). The results of using the Protamex enzyme in APC hydrolysis was shown in Figure 1.

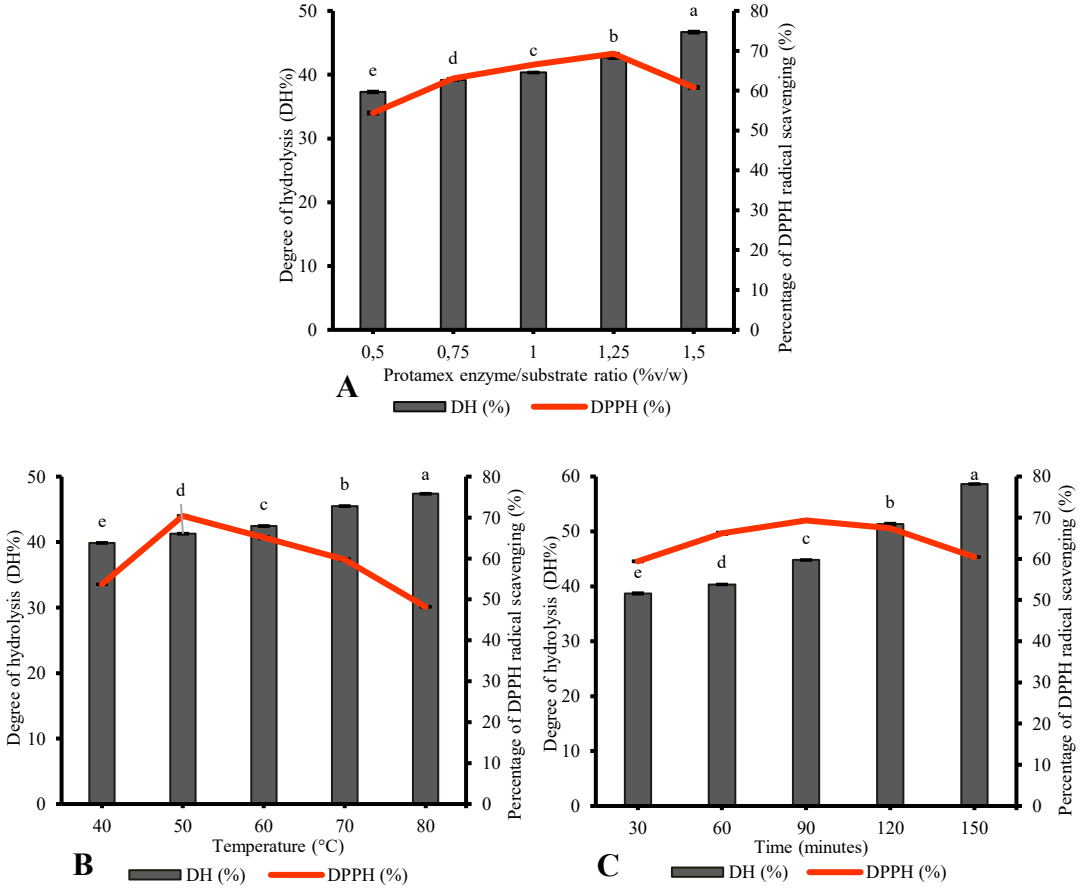

Fig. 1. The effects of hydrolysis conditions of APC by protamex enzyme.

Fig. 1A indicated the effects of Protamex enzyme/substrate ratio (%v/w) APC hydrolysis. Enzymatic hydrolysis disrupts the tertiary structure of proteins, reducing their molecular weight and enhancing peptide interactions with themselves and the environment [18]. Upon addition to the substrate, enzymes adsorb onto its surface, facilitating hydrolysis of peptide bonds susceptible to enzymatic cleavage. Therefore, reaction efficiency peaks when all enzymes are bound to the substrate [19]. ANOVA analysis indicated significant differences among protease-to-substrate ratios surveyed. Increasing enzyme ratio correlates with enhanced

hydrolysis efficiency. At enzyme ratios of 0.5, 0.75, 1, 1.25, and 1.5 (%v/w), hydrolysis efficiencies were 37.29 ± 0.14%, 39.17 ± 0.08%, 40.34 ± 0.08%, 42.69 ± 0.14%, and 46.67 ± 0.15%, respectively (Fig. 1A). Despite the highest hydrolysis efficiency observed at 1.5 (%v/w), this ratio exhibited low radical scavenging activity with 60.80 ± 0.21%. For the objective of DPPH radical scavenging, enzyme ratios of 0.5, 0.75, and 1 (%v/w) exhibited radical scavenging activities of 54.40 ± 0.28%, 63.03 ± 0.08%, and 66.53 ± 0.1%, respectively while 1.25 (%v/w) showed the highest radical scavenging activity at 69.27 ± 0.12%. Therefore, based on DPPH radical scavenging capacity, the chosen enzyme ratio was 1.25 (%v/w).

Fig. 1B illustrated the effects of temperature (°C) on APC hydrolysis. ANOVA analysis indicated that there was variation in the surveyed temperatures (°C). The degrees of hydrolysis were 39.40 ± 0.14%, 41.28 ± 0.08%, 43.16 ± 0.21%, 45.00 ± 0.16%, and 46.91 ± 0.21% at 40, 50, 60, 70, and 80 (°C), respectively. The DH increased with the increase of hydrolysis temperature. The highest DH was at 80 °C, but it also showed the lowest DPPH radical scavenging activity (48.17 ± 0.09%). This could be because APC has fluorescent properties, meaning that although the hydrolysis rate was the highest among the studied temperatures, it was affected by high temperatures. Consequently, the radical scavenging activity was denatured at higher hydrolysis temperatures, leading to poor DPPH radical scavenging capability. The radical scavenging activities at 40, 50, 60, and 70 (°C) were 53.68 ± 0.08%, 70.40 ± 0.04%, 65.11 ± 0.3%, and 59.70 ± 0.23%, respectively. Based on these results and considering DPPH radical scavenging activity as the target function, hydrolysis at 50 °C was the most suitable as it provides the highest radical scavenging activity. According to a study by Zheng X. et al. (2015), hydrolysis at 50 °C achieved a water utilization efficiency of 6.31% [20]. Additionally, Tran Thi Bich Thuy *et al* (2016) produced a protein solution from herring with a high amino acid content using protamex enzyme at 50 °C, achieving a DH of 70.87% [21].

Fig. 1C demonstrated the effects of time (minutes) on APC hydrolysis. The DH was the lowest at 38.70 ± 0.14% at 30 minutes. The DH was time-dependent. The degrees of hydrolysis at 60, 90, 120, and 150 minutes were 40.34 ± 0.08%, 44.80 ± 0.08%, 51.37 ± 0.14%, and 58.64 ± 0.1%, respectively. At a 150-minute hydrolysis period, the DH was high, but the ability to scavenge free radicals was the lowest at 60.45 ± 0.09%. The sample treated for 90 minutes exhibited the highest DPPH free radical scavenging ability (71.25 ± 0.31%) compared to those treated for 120 and 150 minutes, even though the DH was lower at 90 minutes. At 150 minutes, the ability to capture DPPH free radicals was 67.46 ± 0.13% and 60.45 ± 0.09%. Therefore, the 90 minutes of treatment time was the most suitable. This indicated that the hydrolysis time must ensure that enzymes can cleave chemical bonds in the substrate, producing the desired end product, which is the ability to scavenge DPPH radicals. Prolonged hydrolysis allows for complete protein hydrolysis by the enzyme. However, excessive hydrolysis time can create conditions conducive to microbial activity, thereby reducing the ability to scavenge DPPH radicals. According to the study conducted by Palupi N. *et al* (2010), protein hydrolysate was produced from paddy mushrooms (*Volvariella volvaceae*) by incubating the enzyme protamex for 60, 90, and 120 minutes. In a hydrolysis study using brown-striped snapper material, the protein hydrolysate hydrolyzed at 50°C for 90 minutes resulted in a protein yield of 99.45% [22]. According to Khantaphant S. *et al.* (2011), after two hours of reaction, the material's DH was 52.82 ± 0.95% [23].

3.2. Effects of hydrolysis conditions of C-PC by protamex enzyme

C-PC sample was dissolved with 0.01 M phosphate solvent pH = 6. The investigated factors including enzyme percentage used (concentration [E/S] varied from 0.5; 0.75; 1; 1.25; 1.5%v/w), hydrolysis temperature (40, 50, 60, 70, 80 °C) and hydrolysis time (30; 60; 90; 120; 150 minutes). The results are shown in Fig. 2.

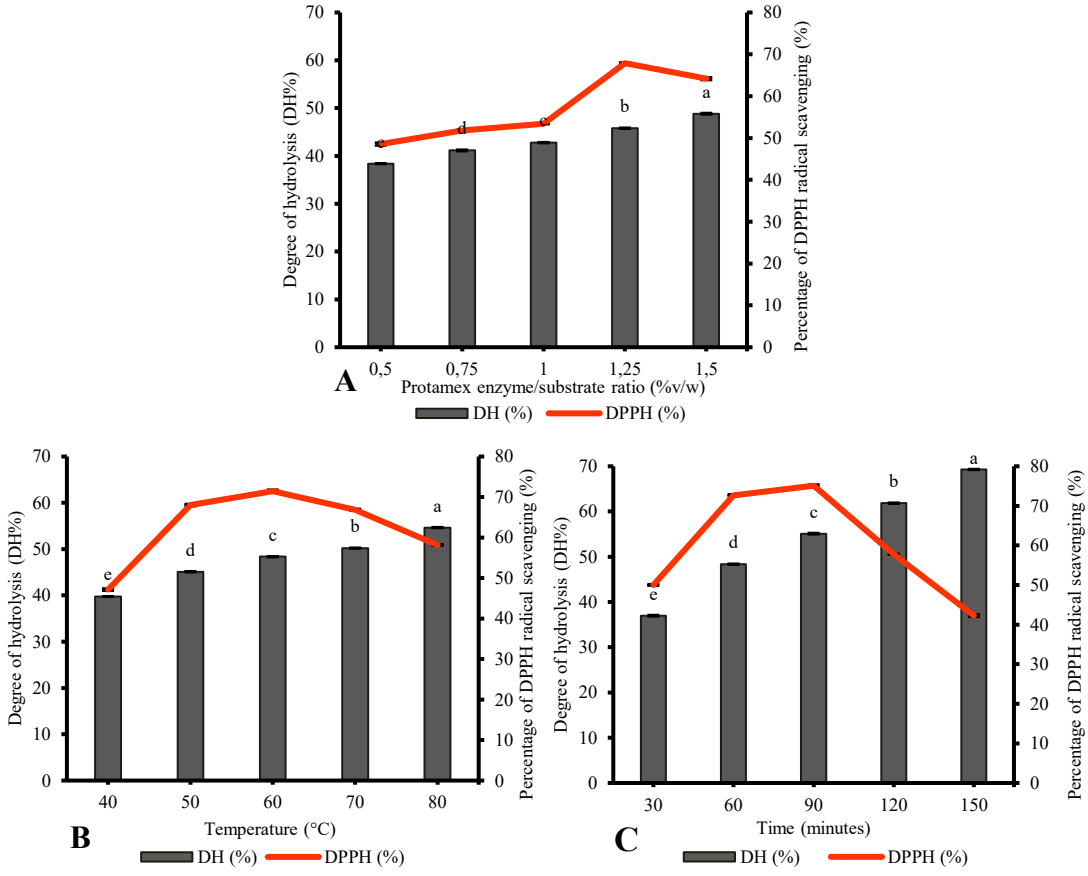

Fig. 2. The effects of hydrolysis conditions of C-PC by protamex enzyme

The effects of the protamex enzyme/substrate ratio (%v/w) on C-PC hydrolysis (Fig. 2A). At an enzyme ratio of 0.5%, both the DH and antioxidant activity was low, only reaching about 38.37 ± 0.14% and 48.55 ± 0.33%, respectively. The enzyme ratio was increased to 0.75%, and both of these indexes increased, with 41.16 ± 0.14% (DH) and 51.82 ± 0.17% (the antioxidant activity). At the enzyme ratio of 1%, the DH and antioxidant activity continued to increase, reaching 42.78 ± 0.08% and 53.44 ± 0.12%, respectively. At an enzyme ratio of 1.25%, the DH continued to rise to 45.81 ± 0.08%, with the antioxidant activity reaching 67.89 ± 0.14%. However, the antioxidant activity of the hydrolysate decreased at an enzyme ratio of 1.5%. This finding echoes similar observations in Truong Thi Mong Thu's study (2022) on the hydrolysis of snakehead fish protein, where increasing protamex enzyme concentration resulted in higher degrees of hydrolysis and antioxidant activity. However, beyond a certain enzyme concentration threshold, both the DH and antioxidant activity declined [24]. The antioxidant potential of bioactive peptides rises as the enzyme-to-substrate ratio approaches an optimum level, facilitating the production of peptides with enhanced activity. Nevertheless, exceeding this optimum ratio can lead to excessive cleavage and other factors that compromise antioxidant activity by altering the peptide's active structure, generating unwanted by-products, and causing various physicochemical changes.

The effects of temperature (°C) on C-PC hydrolysis were shown in Fig. 2B. At a temperature of 40 °C, the DH and antioxidant activity reached about 39.76 ± 0.21% and 47.15 ± 0.26%, respectively. When the temperature increased to 50 °C, both of these indexes increased, with the DH reaching 45.11 ± 0.08% and the antioxidant activity reaching 68.01 ± 0.26%. Next, at a temperature of 60 °C, the DH continued to increase to 48.36 ± 0.08%, and

the antioxidant activity also rose to 71.45 ± 0.35%. However, at temperatures of 70 ℃ and 80 ℃, although the DH still increased, the antioxidant activity decreased, 66.85 ± 0.21% and 58.17 ± 0.17%, respectively. The results were consistent with a study by Liaset B. *et al* (2001) on the enzymatic hydrolysis of salmon bone protein using protamex enzyme [25]. The study investigated temperature milestones ranging from 35 to 70 °C. The findings revealed an increase in degree hydrolysis from 35 to 50 °C, reaching its peak at 50 °C. At temperatures higher or lower than the optimum temperature for the enzyme, the DH increased while the ability to cleave peptide bonds decreased.

The effects of time (minutes) on C-PC hydrolysis were presented in Fig. 2C. At 30 minutes, both the DH and antioxidant activity were quite low, only reaching about 36.97 ± 0.14% and 50.03 ± 0.17%, respectively. However, at 60 minutes and 90 minutes, both of these indexes increased to 48.36 ± 0.08% (DH) and 55.11 ± 0.14% (antioxidant activity). At 120 minutes and 150 minutes, the DH did not change significantly, remaining high at 61.85 ± 0.08% and 69.29 ± 0.08%. However, the antioxidant activity decreased slightly as the treatment time increased, reaching 57.77 ± 0.08% and 42.28 ± 0.31%, respectively. The tendency in the current study was in line with the findings of Truong Thi Mong Thu *et al* (2022). They investigated hydrolysis times ranging from 18 to 36 hours. From 18 to 24 hours, the DH gradually increased. However, between 30 and 36 hours, the DH showed a gradual decrease [24]. This decline could be attributed to the prolonged exposure of protein molecules to enzymes over time. Initially, longer hydrolysis times facilitate increased enzyme activity in cleaving peptide bonds within the proteins, thereby raising the DH (DH). However, beyond a certain point, extensive cleavage of protein molecules may occur, potentially depleting or deactivating the enzyme. This depletion or deactivation could contribute to the observed decrease in hydrolysis activity during this extended period.

3.3. Effects of hydrolysis conditions of R-PE by protamex enzyme

The effects of enzyme/substrate ratios (0.5; 0.75; 1; 1.25; 1.5, %v/w), hydrolysis temperatures (40, 50, 60, 70, 80 °C) and hydrolysis times (30; 60; 90; 120; 150 minutes) on R-PE hydrolysis were shown in Fig. 3.

The DH changed with the concentration concentration-dependent enzyme, increasing from 32.17 ± 0.82% to 75.94 ± 0.59%. The results showed that when hydrolysing proteins with enzymes at different concentrations, the antioxidant capacity also changed. When the enzyme ratio increased from 0.5% to 1%, the DPPH radical of hydrolysate increased from 43.83 ± 0.51% to 64.40 ± 0.54% (Fig. 3A). If the enzyme concentration continues to increase, the number of these peptides will increase, but only to a certain value. The hydrolysis process happened better, but at the same time, it also cleaved bioactive peptides into peptides of smaller sizes and free amino acids. The potency of the hydrolysate's DPPH radical scavenging activity depends on the size of peptides and the type of enzyme [26]. Therefore, it reduced the antioxidant capacity of the hydrolysate. Other studies indicated that most enzymatic digestion sites are located next to hydrophobic amino acids, as Arise A. *et al* (2016) reported that lower-molecular weight peptides demonstrated greater efficacy in scavenging DPPH radicals, with a negative correlation observed between molecular weight and DPPH scavenging rates, as also supported by the results of this study [27]. As shown in Fig. 3A, a suitable enzyme/substrate ratio of 1%v/w was selected for subsequent experiments.

The highest DPPH radicals value was 65.09 ± 0.14% at 50 ºC. The DH of hydrolysate using protamex enzyme reached the highest value at 80ºC and DPPH capacity 42.78 ± 0.64% (Fig. 3B). In temperature values which were higher or lower than the optimal temperature value of the enzyme, the DH rose, and the DPPH of the hydrolysate decreased. A similar observation was reported in the study of Vo H. *et al (*2023); pea protein concentrate was hydrolyzed to obtain bioactive peptides and amino acids. DH significantly increased ($p < 0.05$)

with the increasing temperature [28]. Consequently, a suitable temperature of 50 ℃ was selected for subsequent experiments.

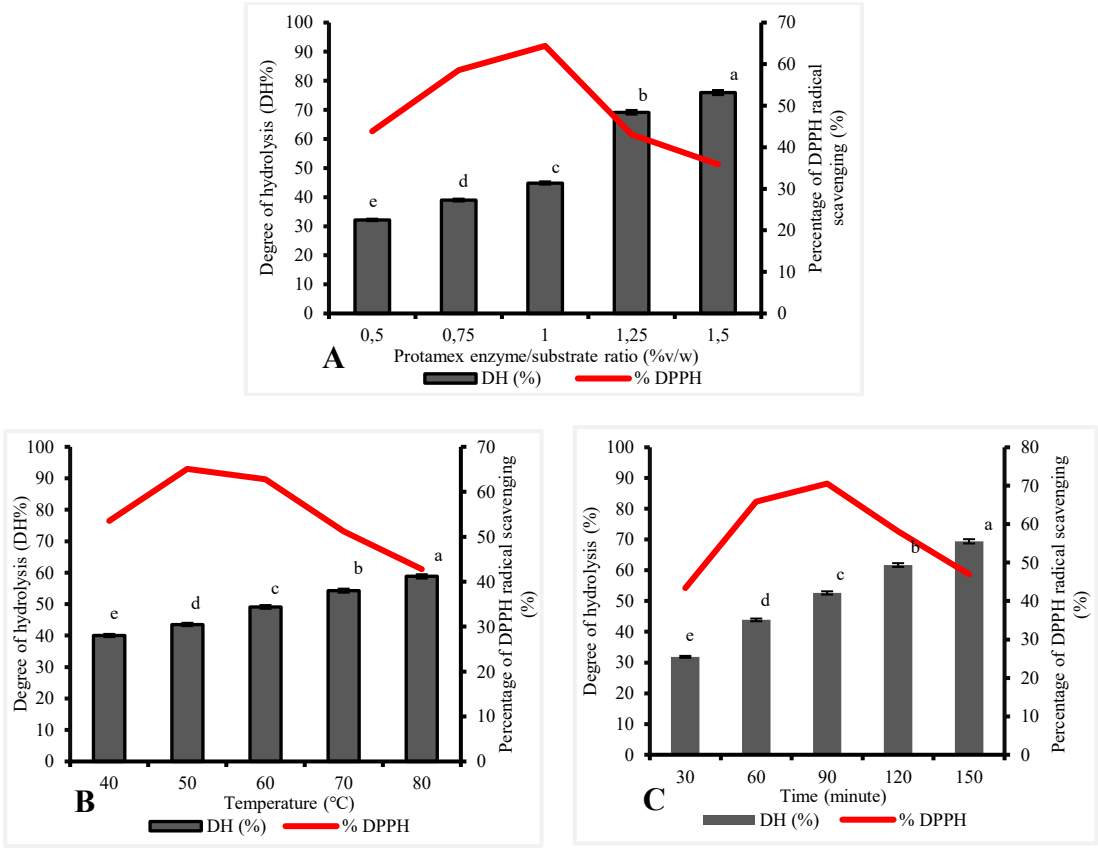

Fig. 3. The effects of hydrolysis conditions of R-PE by protamex enzyme.

The DH gradually increased over time. When the hydrolysis time increased from 30 minutes to 150 minutes, the DH increased from 29.28 ± 0.18% to 79.33 ± 0.65%. Thus, the suitable hydrolysis time to obtain the biological activity of the peptide preparation was 60 minutes. At this time, the antioxidant capacity of the peptide preparation using protamex enzyme was 67.94 ± 0.48%. Both the DH and DPPH increased as the reaction time increased, increasing rapidly within the initial 90 minutes of reaction (Fig. 3C). Zeng X. *et al* (2015) reported a similar DH profile of corn glutelin with reaction time. The DH of the protein obtained was 6.31%, respectively, at a hydrolysis time of 150 min. In particular, the activity of hydrolysates (58.86 ± 1.40%) at a hydrolysis time of 120 min was prominently higher than that. ($p < 0.05$) [20]. Based on the DPPH radical scavenging activity and DH of R-PE hydrolysis, the suitable pretreatment conditions were constructed at 1 %v/w, 50 ℃ and 90 min.

4. CONCLUSION

In this study, the effects of protamex enzyme/substrate ratio, temperature, and time on the DH and free radical scavenging ability of APC, C-PC, and R-PE hydrolysates extracted from *C. aerea* were investigated. The results indicated that these factors significantly influenced the DH and antioxidant activity of APC, C-PC, and R-PE hydrolysates. Hydrolysis of APC at an enzyme/substrate ratio of 1.25% (v/w) and a temperature of 50°C for 90 minutes yielded a DPPH free radical scavenging ability of 71.25%, with a corresponding DH of

44.80%. Under the same conditions, C-PC exhibited a DPPH free radical scavenging ability of 72.67%, with a DH of 48.36%. Similarly, hydrolyzing R-PE at an enzyme/substrate ratio of 1.25% (v/w) and a temperature of 50 °C for 90 minutes resulted in a DPPH free radical scavenging ability of 70.56%, accompanied by a DH of 52.63%. These results suggest the potential use of these hydrolysates as natural antioxidants in various applications.

Acknowledgements: This study was sponsored and funded by Ho Chi Minh City University of Industry and Trade, Contract No. 18/HD-DCT dated 9 January 2024.

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TÓM TẮT

HOẠT TÍNH KHÁNG OXY HÓA CỦA DỊCH THỦY PHÂN PHYCOBILIPROTEIN TỪ RONG *Chaetomorpha aerea* BẰNG ENZYME PROTAMEX

Hoàng Thị Ngọc Nhơn*, Hồ Xuân Quỳnh, Phan Quỳnh Đoan, Hồ Thanh Thưởng

Trường Đại học Công Thương Thành phố Hồ Chí Minh

*Email: *nhonhtn@huit.edu.vn*

Allophycocyanin (APC), C-phycocyanin (C-PC) và R-phycoerythrin (R-PE) là các phycobiliprotein, được chú ý nhờ nhiều ứng dụng công nghệ sinh học trong công nghệ thực phẩm, mỹ phẩm, quy trình phân tích và được sử dụng rộng rãi làm chất đầu dò huỳnh quang. Chúng là sắc tố có mặt chủ yếu ở tảo đỏ và tảo xanh. Trong nghiên cứu này, chúng tôi tập trung nghiên cứu các yếu tố ảnh hưởng đến quá trình thủy phân APC, C-PC và R-PE từ *Chaetomorpha aerea* bằng enzyme protamex để thu được peptide có hoạt tính sinh học. Các yếu tố ảnh hưởng được khảo sát như: nồng độ enzyme, nhiệt độ và thời gian thủy phân. Với tỷ lệ enzyme/cơ chất 1,25% v/w, ở 50 °C trong 90 phút, cho ra kết quả khả năng bắt gốc tự do DPPH với mức độ thủy phân đối với APC, C-PC và R-PE lần lượt là (71,25%; 44,80%); (72,67%; 48,36%) và (70,56%; 52,63%).

Từ khoá: Allophycocyanin, C-phycocyanin, R-phycoerythrin, *Chaetomorpha aerea,* enzyme protamex.