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AKADÉMIAI KIADÓ

Effect of hydrolytic degree on antioxidant activity and functional properties of *Acetes japonicus* proteolysate

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ABSTRACT

This study aims to examine the effect of hydrolysis degree (DH) on both antioxidant activity and functional properties of *Acetes japonicus* proteolysate (AP). Consequently, the AP showed the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (SA) and ferric reducing antioxidant power (FRAP) at DH of 66.7%. Whereas, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical ($ABTS^{\bullet+}$) SA and superoxide anion radical ($O_2^{\bullet-}$) SA of the AP peaked at DH of 75.8%. In addition, its strongest Fe^{2+}-chelating rate was found at DH of 72.1%. In the pH range from 3 to 8, the AP showed solubility over 55% even after heat treating, foaming capacity (FC) of 5.7–80.0%, foaming stability (FS) of 2.9–77.0%, emulsifying-activity index (EAI) of 16.1–56.3 $m^2\,g^{-1}$, and emulsifying stability index (ESI) of 12.4–156.7 min. The highest water-holding capacity (WHC) and oil-holding capacity (OHC) of the AP were observed at DH of 66.7% and 50.6%, respectively. This study enhanced value of the *Acetes* by producing antioxidant AP possessing functionalities.

KEYWORDS

***Acetes japonicus*, antioxidant activity, hydrolysis degree, functional property, protein hydrolysate**

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1. INTRODUCTION

Biological antioxidant defence systems can fail to protect the body against reactive radicals, triggering numerous oxidative stress-related diseases (Halim et al., 2016). In the food industry, by releasing undesirable secondary peroxides due to lipid oxidation, free radicals shorten the shelf life and reduce the quality of food products (Putra et al., 2018). These negative effects could be prevented by using natural antioxidants. Of which, protein hydrolysates from aquatic products/by-products were considered as safe, healthy, and easily-absorbed (Intarasirisawat et al., 2012). Enzymatic hydrolysis has been an efficient method to improve both antioxidant activity and functionalities of protein hydrolysate while maintaining their nutritional value (Noman et al., 2018).

Recently, endoproteases have been applied to obtain bioactive peptides or protein hydrolysates from sole skin gelatine and halibut skin (Sila and Bougatef, 2016) and salmon frame (Siddik et al., 2021). Flavourzyme® 500 MG preparation from *Aspergillus oryzae*, displaying both exo- and endopeptidase activities, probably covering a broad range of substrate, was also used to generate antioxidant protein hydrolysate from tuna liver (Sila and Bougatef, 2016), flounder by-products (Siddik et al., 2021), and small shrimp (Vo et al., 2021a). In addition, this preparation was utilised to gain other bioactive protein hydrolysates with calcium-binding (Vo et al., 2018), iron-binding (Vo et al., 2020), and copper-binding capacity (Vo et al., 2021b) from small shrimps.

DH, the proportion of peptide bonds being cut in hydrolysate, is one of the most crucial factors that remarkably impacts the size, composition, and amino acid sequence of peptides, as a result, affecting the antioxidant activity and functional properties of protein hydrolysate (Li et al., 2014). However, there have not been many publications on the effect of DH on both antioxidant activity and functional properties of the AP so far.

2. MATERIALS AND METHODS

2.1. Materials

The *Acetes* from Ninh Thuan province with the protein content of 72.8 ± 0.7% (dry weight) was used in this study.

Flavourzyme® 500 MG was obtained from Novozymes (Bagsvered, Denmark). Chemicals of analytical grade were purchased from Sigma–Aldrich and Merck. Double-distilled water was used in the experiments.

2.2. Methods

2.2.1. Preparation of AP. The procedure of Vo et al. (2020) with slight modification was performed to prepare the AP. After adding water into the ground *Acetes* with the ratio of 8:1 (v/w), the mixture was heated at 90 °C for 10 min to deactivate endogenous enzymes. 1M NaOH or 1M HCl solution was used to set the pH value at 7. After that, before hydrolysing at 55 °C, Flavourzyme was added with the ratio of 60 U g^{-1} protein. The enzyme was then deactivated by heating the hydrolysates for 10 min at 90 °C after the hydrolysis time varied from 0 to 180 min.

The obtained supernatants collected by centrifuging the hydrolysates, were freeze-dried and stored at −20 °C until used.

2.2.2. Determination of hydrolysis degree. The DH of the AP was determined employing the method of Intarasirisawat et al. (2012).

2.2.3. Antioxidant assays. The AP, vitamin C, and Na_2EDTA solutions were prepared at the concentration of 1 mg ml^{-1} before testing their antioxidant activity. DPPH SA, FRAP value, $O_2^{\bullet-}$ SA, $ABTS^{\bullet+}$ SA, and Fe^{2+}-chelating rate of the AP were determined using methods described by Vo et al. (2021a).

2.2.4. Determination of functional properties. Solubility, heat stability, foaming property, emulsifying property, OHC, and WHC of the AP were examined by the methods described by Vo et al. (2020).

2.2.5. Statistical analysis. Data were presented as means ± standard deviations of triplicate determinations. Analysis of variance (one-way ANOVA) was performed on the data using the Statgraphics Centurion 18 software, and the significance was determined using Tukey's method ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Effect of hydrolysis time on DH of the AP

In the first 40 min of hydrolysis, DH of the AP remarkably increased from 0 to 65.9 ± 2.9% (Fig. 1), which could be because of the fact that a large quantity of *Acetes* protein is broken down into small peptides due to the Flavourzyme preparation, a mixture of endo- and exoproteases, having a broad specificity (Vo et al., 2020). Consequently, with the decreased amount of substrate matching to active site of the Flavourzyme, DH value changed slowly in the latter stage of hydrolysis. Similar result was observed in the publication of Noman et al. (2018). For further studies, DH values of 0%, 50.6%, 66.7%, 69.7%, 71.5%, 72.1%, and 75.8% were chosen.

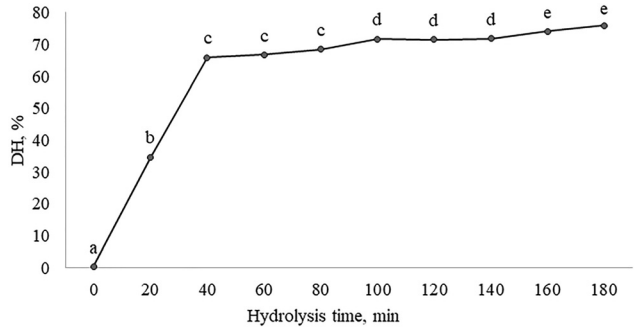

Fig. 1. Effect of hydrolysis time on hydrolysis degree (DH) of *Acetes japonicus* proteolysate (AP). Different letters indicate significant differences ($P < 0.05$)

3.2. Effect of DH on antioxidant activity of the AP

It is shown in Fig. 2A and Fig. 2B that DPPH SA and FRAP values of the AP were directly proportional to its DH from 0 to 66.7% and peaked at 22.5 ± 0.3% (1.8 times lower than that of vitamin C) and 74.6 ± 1.9 μM TE (24.2 times lower than that of vitamin C), respectively, at DH 66.7%. At low DH value (DH < 66.7%), the low antioxidant activity of the AP may be due to the presence of a

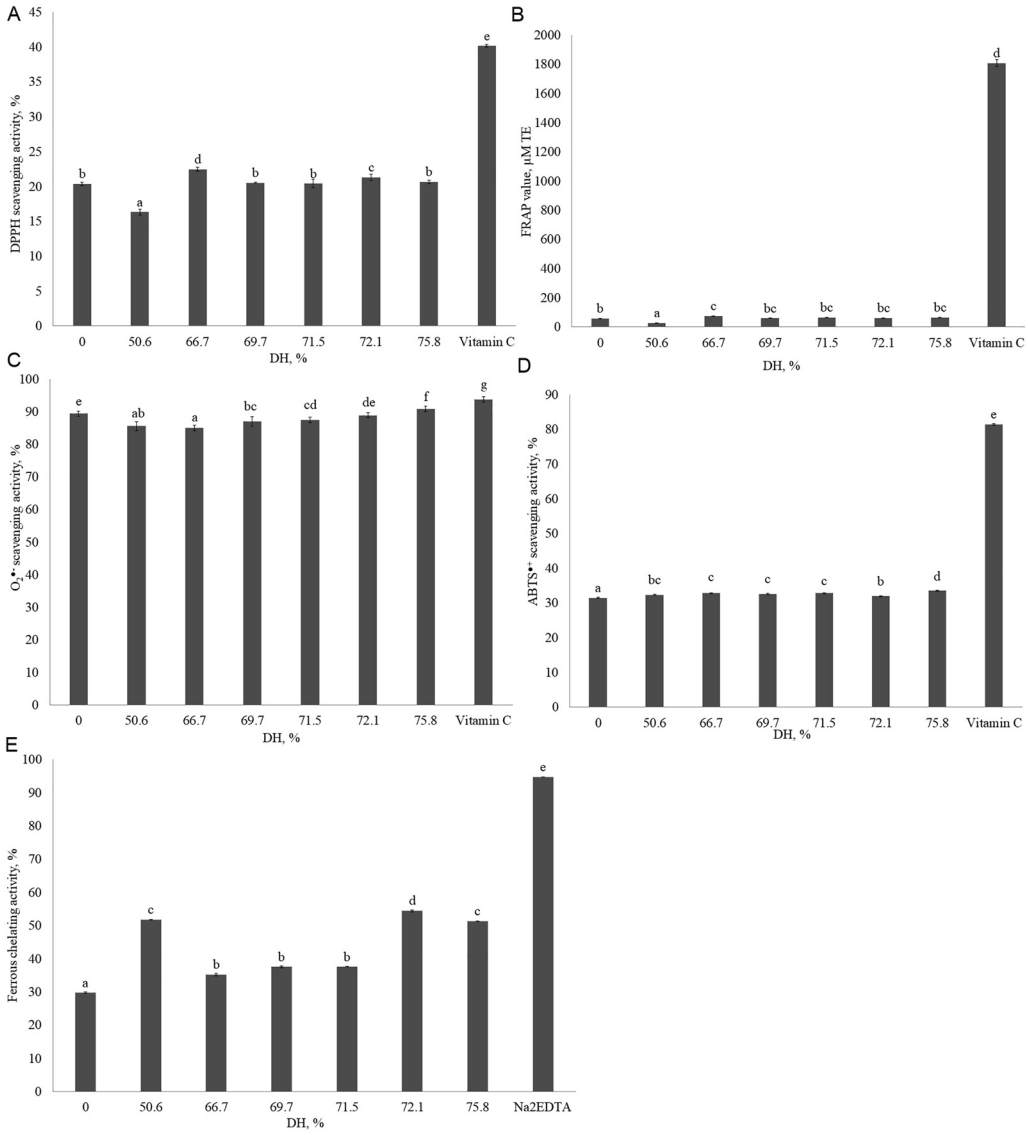

Fig. 2. Effect of DH on DPPH SC (A), FRAP value (B), $O_2^{\bullet-}$ SC (C), $ABTS^{\bullet+}$ SC (D), and Fe^{2+}-chelating activities (E) of AP. Bars with different letters indicate significant differences ($P < 0.05$)

low amount of electron donor groups (e.g, Glu, Asp, etc. (Charoenphun et al., 2013)), a large amount of long chain peptides, or non-hydrolysed proteins with bulky spatial structures and inter- and intramolecular bonds in the protein hydrolysate (Noman et al., 2018). On the other hand, at DH 66.7%, *Acetes* protein was almost hydrolysed into low molecular weight peptides that could easily move to free radicals (Vo et al., 2021a). DPPH SA and FRAP values significantly decreased by continuously increasing DH to 75.8%, probably resulting from the lower antioxidant activity expressed from a large amount of free amino acids in high DH AP because of the lack of structural resonance (Intarasirisawat et al., 2012). You et al. (2009) also reported similar observations.

Both $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ SA reached the maximum value of 33.5 ± 0.1% (2.4-fold lower than that of vitamin C) and 90.8 ± 0.8% (1.1 times lower than that of vitamin C) at DH 75.8% (Fig. 2C and Fig. 2D). The antioxidant activity of the AP was enhanced as high DH AP predominantly encompassed low molecular weight peptides, exposing more H-donating groups such as Tyr's phenol and His's imidazole ring than high molecular weight peptides did (Li et al., 2014). This study showed the same result with the previous researches of You et al. (2009) and Li et al. (2014).

It is shown in Fig. 2E that when elevating DH from 0 to 72.1%, Fe^{2+}-chelating activity of the AP augmented and at DH 72.1%, it reached the peak of 54.5 ± 0.3% (1.7 times lower than that of Na_2EDTA). During protein hydrolysis, Fe^{2+}-chelating activity of the AP was enhanced due to the release of small peptides from intact proteins, possessing amino acid side chains (Ser, Thr, Lys, Gln, etc.) able to chelate Fe^{2+} ions (Vo et al., 2020). However, a decrease in Fe^{2+}-chelating activity was observed when DH achieved 75.8%, which could be understood as the smaller fragments hydrolysed from bioactive peptides were incompetent to coordinate with Fe^{2+} (Intarasirisawat et al., 2012).

3.3. Effect of DH on functional properties of the AP

3.3.1. Solubility. This study showed a directly proportional relationship between DH in range of 0–72.1% and solubility of AP (Fig. 3A). The solubility of AP is increased by favouring the formation of hydrogen bonds with water molecules, exposing more charged and polar side chains (Li et al., 2012). At DH 75.8%, there was, however, a decrease in solubility. It may be due to the fact that Flavourzyme, usually producing proteolysate with high concentration of hydrophobic amino acids, lead to protein aggregation via hydrophobic interactions (Vo et al., 2020).

One of the important factors in the solubility of the proteolysate is the pH, as by altering the charge on the weakly acidic and basic side-chain groups, when maximally charged, AP generally exhibits the highest solubility (Vo et al., 2020). In this study, at various DH values, the highest solubility, over 80%, was found for AP at pH 8 (Fig. 3A), which was 1.03–1.22 times higher than that of pink perch proteolysates (Noman et al., 2018). High solubility in alkaline pH was because protein aggregation was prevented due to the number of negative charge residues, enhancing repulsive forces of peptides in AP (Santos et al., 2011). With a high solubility in a broad pH range, the AP could be potentially useful in a variety of food formulations.

3.3.2. Heat stability. It was revealed by Li et al. (2012) that during heat treatment, protein aggregation resulted from poor balance between hydrophobic and hydrophilic forces in the proteolysate. Besides, the conformation of peptide could be altered by heat, leading to aggregation caused by hydrophobic interaction (Vo et al., 2020). Moreover, the ionisation of peptides in the proteolysate could be generated by solution pH, promoting ion repulsion preventing protein aggregation. In general, at pH 3–8, the solubility of the AP stayed above 55% after heating at 63 °C for 30 min or 93 °C for 30 s at all DH values (Fig. 3B and 3C).

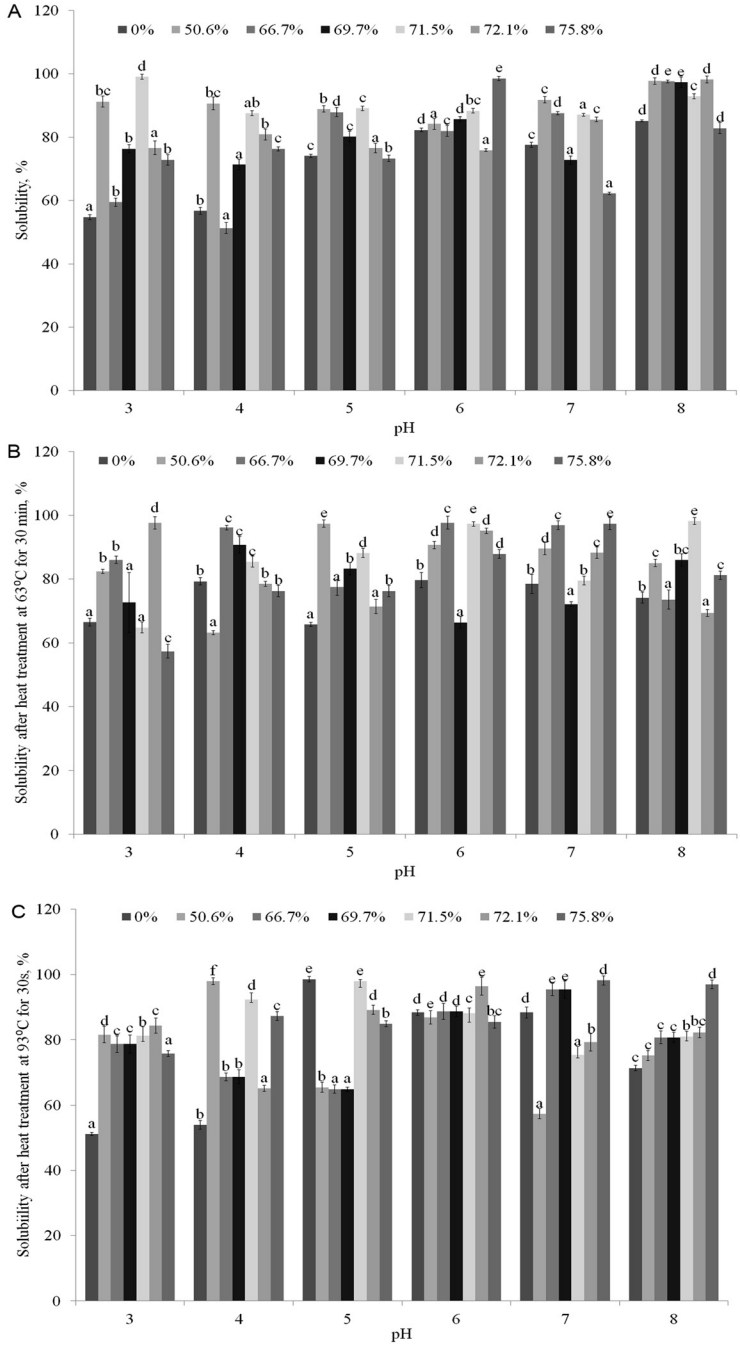

Fig. 3. Effect of DH on solubility (A) and heat stability (B and C) of AP. The same bars with different letters indicate significant differences ($P < 0.05$)

3.3.3. Foaming property. As shown in Fig. 4, there was a negative relationship between DH and foaming property of the AP. The small peptides showed poor surfactant activity and inability to stabilise the air cells of the foam, despite their great speed to the foam surface

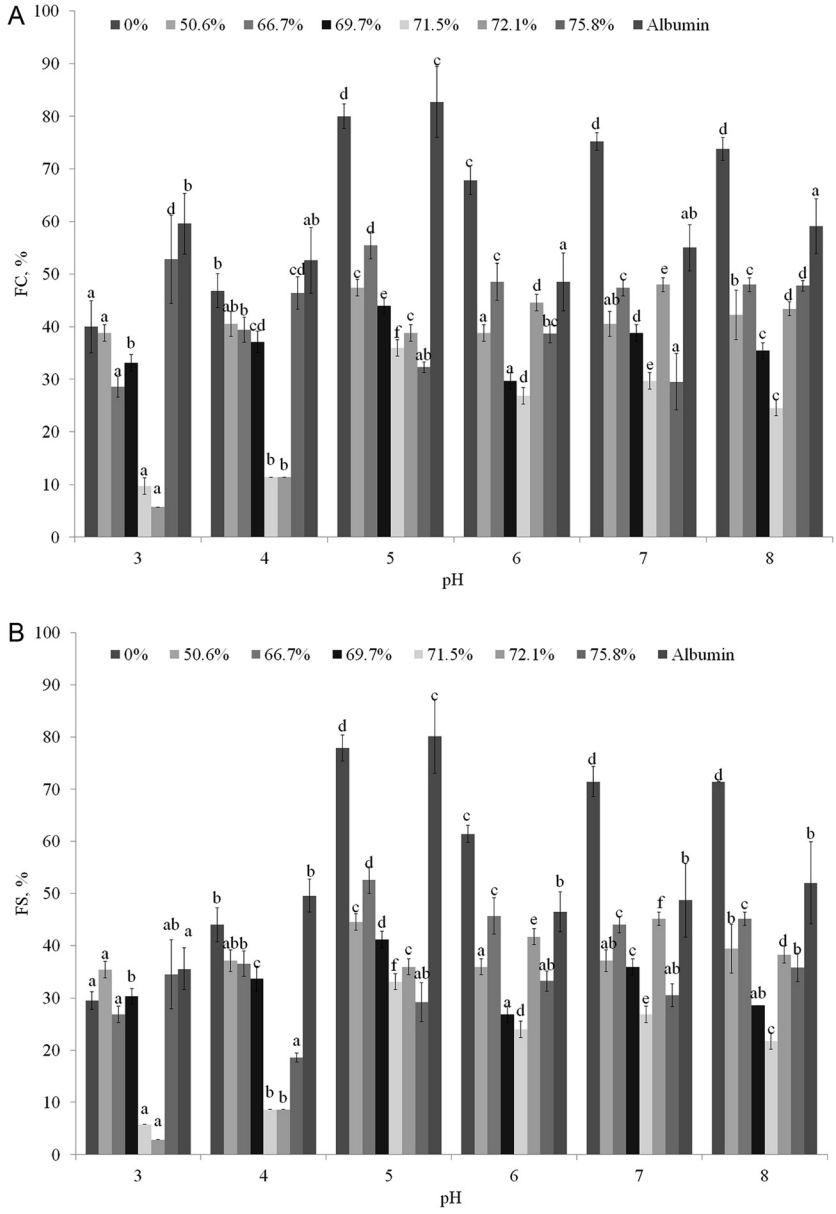

Fig. 4. Effect of DH on FC (A) and FS (B) of AP. The same bars with different letters indicate significant differences ($P < 0.05$)

(Vo et al., 2020), lowering both FC and FS of the high DH proteolysate. Conversely, the foaming property of the AP could be improved by thick and strong films surrounding air bubbles, which were merely achieved by long-chain peptides or partially hydrolysed protein (Halim et al., 2016).

The pH had an effect on foaming property of the AP via changing net charge of peptides, the absorption of peptides at water-air interface, nature of the film, and protein-protein interaction within the matrix (Vo et al., 2020). In the pH range of 3–8, FC and FS of the AP with various DH were in range 5.7–80.0% and 2.9–77.0%, which were 1.1–14.5-fold and 1.1–40.3 times lower

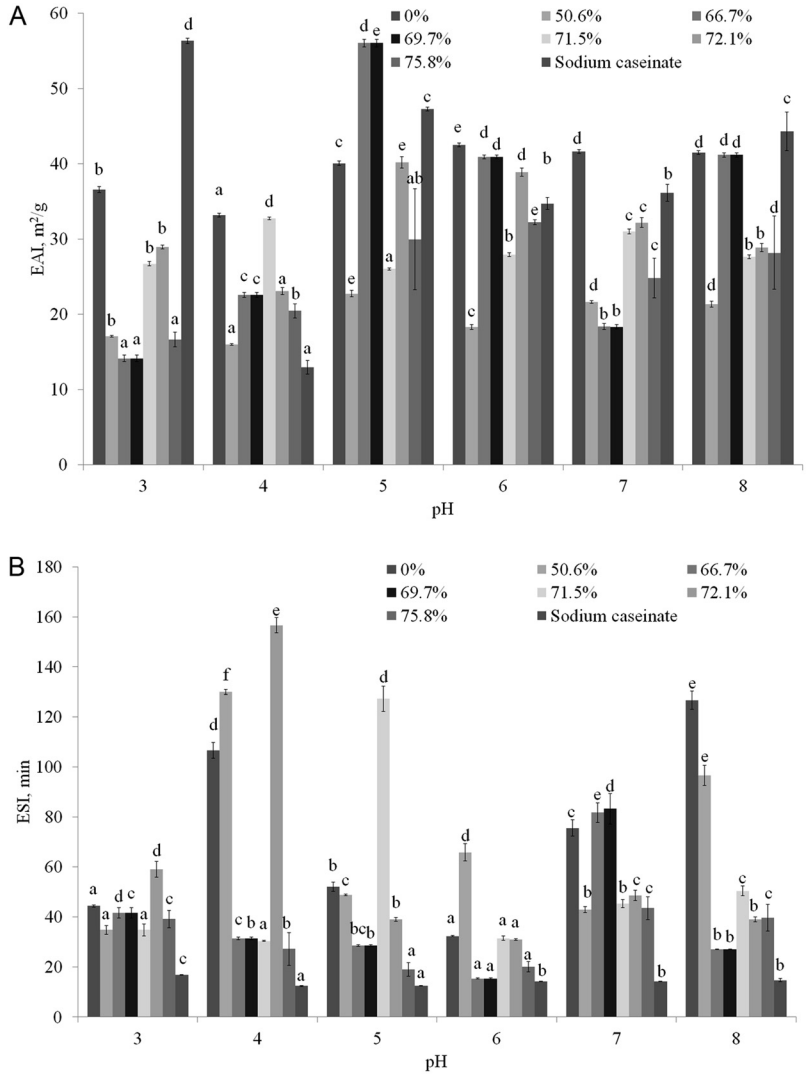

Fig. 5. Effect of DH on emulsifying-activity index (EAI) (A) and emulsifying stability index (ESI) (B) of AP. The same bars with different letters indicate significant differences ($P < 0.05$)

than those of albumin, respectively (Fig. 4). Generally, the AP could be used as foaming agent in a broad range of food products.

3.3.4. Emulsifying property. By affecting the characteristics of peptide such as molecular weight, surface property, and hydrophobicity, DH plays a key role in emulsifying property of the AP (Santos et al., 2011). Small peptides could rapidly migrate to and be adsorbed at the oil-water interface, however, they are unable to unfold and reorient at the interface as large peptides, leading to their less efficiency in reducing the interfacial tension (Li et al., 2012). Also, the tertiary structure of protein that provides thick and durable films surrounding oil droplets and steric effect is lost due to excessive hydrolysis, resulting in weak interfacial film surrounding oil droplets (Halim et al., 2016). Hence, when increasing DH value as illustrated in Fig. 5, a poorer EAI and ESI could be observed.

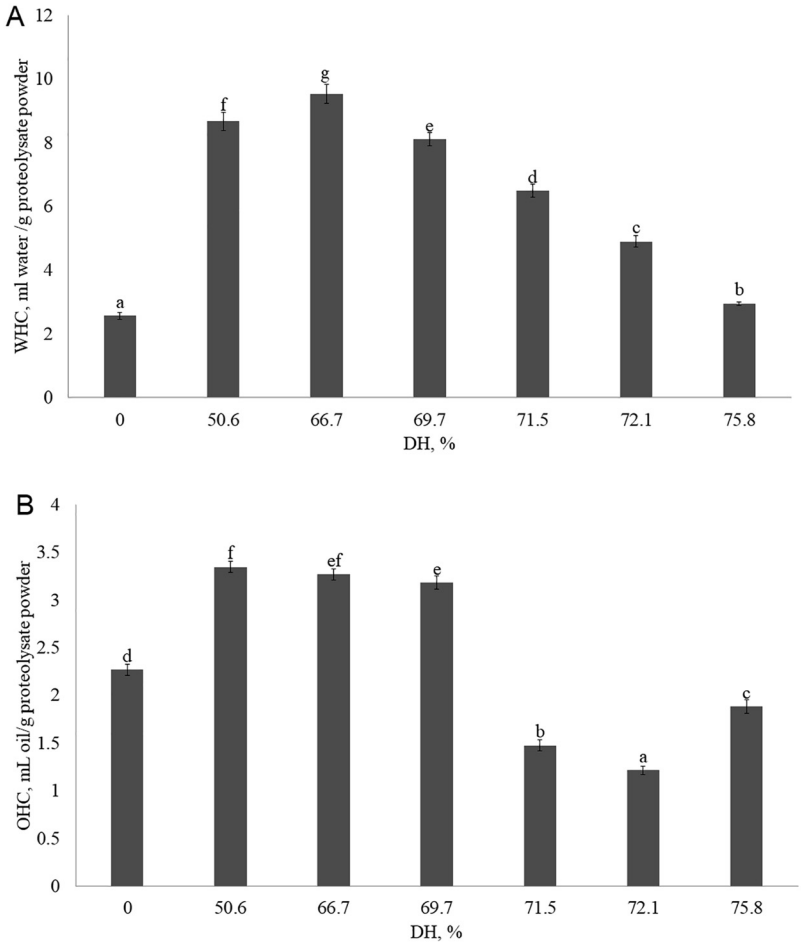

Fig. 6. Effect of DH on water-holding capacity (WHC) (A) and oil-holding capacity (OHC) (B) of AP. The same bars with different letters indicate significant differences ($P < 0.05$)

Besides, through altering peptide surface hydrophobicity and protective peptide layer, pH also strongly affects the emulsifying property of the AP (Halim et al., 2016). In this study, at pH 3–8, EAIs of those various DH APs were 1.3–3.9-fold lower compared to that of sodium caseinate, while their ESIs were 1.4–9.3 times higher than that of sodium caseinate. Therefore, they can be assumed to be able to provide desirable emulsifying properties in the preparation of mayonnaise and salad dressings.

3.3.5. WHC. WHC of the AP was directly proportional to DH in the range 0–66.7% and achieved the maximum value of 9.5 ± 0.3 mL water/g AP powder (Fig. 6A), which was 10.5 times higher than that of golden apple snail proteolysate (Putra et al., 2018). Vo et al. (2020) proposed that smaller peptides were often more hydrophilic, resulting in being more effective in keeping water than larger peptides. Nevertheless, as DH value increased to 75.8%, there was a significant decrease in WHC (Fig. 6a), which was probably because extensive hydrolysis lowers the ability of protein to entrap water (Halim et al., 2016). It is then concluded that the AP might be used as a moisture-keeping agent for food products.

3.3.6. OHC. OHC of the AP reached the maximum of 3.4 ± 0.1 mL oil/g AP powder at DH of 50.6% (Fig. 6B), which was 1.3 times higher than that of Chinese sturgeon proteolysate (Noman et al., 2018). The access of oil particle to internal aliphatic side chains was favoured, boosting OHC of the AP when proteolysis opened protein conformational structures (tertiary and quaternary) (Vo et al., 2020). Nevertheless, a large amount of short-chain peptides possessing superior hydrophilicity in high DH proteolysate (DH>50.6%) decreases the interaction between peptide and lipid, reducing OHC (Noman et al., 2018). Hence, the AP powder might be utilised to retard phase separation as well as improve palatability and taste retention of some food products (Santos et al., 2011).

4. CONCLUSIONS

This study is the very first to investigate the effect of DH on both antioxidant activity and functional properties of protein hydrolysate from the small shrimp. Using 5 different assays for testing the antioxidant activity, the research provides preliminary data for the application of the AP in food and pharmaceutical products. This proves the added value of the *Acetes*, a cheap aquatic raw material source in Vietnam.

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REFERENCES

Charoenphun, N., Cheirsilp, B., Sirinupong, N., and Youravong, W. (2013). Calcium-binding peptides derived from tilapia (*Oreochromis niloticus*) protein hydrolysate. *European Food Research and Technology*, 236(1): 57–63.

Halim, N.R.A., Yusof, H.M., and Sarbon, N.M. (2016). Functional and bioactive properties of fish protein hydolysates and peptides: a comprehensive review. *Trends in Food Science & Technology*, 51: 24–33.

Intarasirisawat, R., Benjakul, S., Visessanguan, W., and Wu, J. (2012). Antioxidative and functional properties of protein hydrolysate from defatted skipjack (*Katsuwonous pelamis*) roe. *Food Chemistry*, 135(4): 3039–3048.

Li, X., Luo, Y., Shen, H., and You, J. (2012). Antioxidant activities and functional properties of grass carp (*Ctenopharyngodon idellus*) protein hydrolysates. *Journal of the Science of Food and Agriculture*, 92(2): 292–298.

Li, X., Shen, S., Deng, J., Li, T., and Ding, C. (2014). Antioxidant activities and functional properties of tea seed protein hydrolysates (*Camellia oleifera Abel.*) influenced by the degree of enzymatic hydrolysis. *Food Science and Biotechnology*, 23(6): 2075–2082.

Noman, A., Xu, Y., AL-Bukhaiti, W.Q., Abed, S.M., Ali, A.H., Ramadhan, A.H., and Xia, W. (2018). Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (*Acipenser sinensis*) by using papain enzyme. *Process Biochemistry*, 67: 19–28.

Putra, S.N.K.M., Ishak, N.H., and Sarbon, N.M. (2018). Preparation and characterization of physico-chemical properties of golden apple snail (*Pomacea canaliculata*) protein hydrolysate as affected by different proteases. *Biocatalysis and Agricultural Biotechnology*, 13: 123–128.

Santos, S.D.A.D., Martins, V.G., Salas-Mellado, M., and Prentice, C. (2011). Evaluation of functional properties in protein hydrolysates from bluewing searobin (*Prionotus punctatus*) obtained with different microbial enzymes. *Food and Bioprocess Technology*, 4(8): 1399–1406.

Siddik, M.A.B., Howieson, J., Fotedar, R., and Partridge, G.J. (2021). Enzymatic fish protein hydrolysates in finfish aquaculture: a review. *Reviews in Aquaculture*, 13(1): 406–430.

Sila, A. and Bougatef, A. (2016). Antioxidant peptides from marine by-products: Isolation, identification and application in food systems. A review. *Journal of Functional Foods*, 21: 10–26.

Vo, T.D.L., Pham, K.T., Bui, N.H.Y., Luong, N.N.Y., Nguyen, N.T., Mai, C T., and Nguyen, A.H. (2021a). Identification of a new antioxidant peptide from protein hydrolysate of *Acetes japonicus*. *Proceeding of the 5th International Conference on Chemical Engineering, Food and Biotechnology - ICCFB2021*: pp. 58–66.

Vo, T.D.L., Pham, K.T., and Doan, K.T. (2021b). Identification of copper-binding peptides and investigation of functional properties of *Acetes japonicus* proteolysate. *Waste and Biomass Valorization*, 12: 1565–1579.

Vo, T.D.L., Pham, K.T., Le, L.T., and Nguyen, T.T.H. (2018). Identification of a new calcium-binding peptide from enzymatic proteolysate of *Acetes japonicus*. *Journal of Food Processing and Preservation*, 42(12): e13837.

Vo, T.D.L., Pham, K.T., Le, V.M.V., Lam, H.H., Huynh, O.N., and Vo, B.C. (2020). Evaluation of iron-binding capacity, amino acid composition, functional properties of *Acetes japonicus* proteolysate and identification of iron-binding peptides. *Process Biochemistry*, 91: 374–386.

You, L., Zhao, M., Cui, C., Zhao, H., and Yang, B. (2009). Effect of degree of hydrolysis on the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates. *Innovative Food Science and Emerging Technologies*, 10: 235–240.