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# Effect of different molecular weight on the antioxidant activity and physicochemical properties of golden apple snail (*Ampullariidae*) protein hydrolysates

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**Abstract**

Golden apple snail (GAS) is reported to have a medicine effect for treat broken bones, back pain and stool bloody. This study was aimed to investigate the fractionation of protein hydrolysate from the golden apple snail (GAS) using an ultrafiltration technique with molecular weight cut-offs of 3, 5, and 10 kDa, followed by the determination of its antioxidant activity and functional properties. In terms of antioxidant activity, fraction of 3 kDa showed the highest reducing power and DPPH radical scavenging activity. Additionally, fraction of 5 kDa had the highest chelating effect on ferrous ion (85.6%). The results also revealed that fraction of 3 kDa showed good emulsion activity index and water binding capacity, while 5 kDa showed good foaming capacity and solubility. These results suggested that GASH showed potential for application in the food industry as its antioxidant, with strong functional properties able to be obtained by varying its molecular weights.

## 1. Introduction

An antioxidant is a molecule or compound which is able to inhibit the oxidation of a given substrate (Chalamaiah *et al.,* 2012). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are synthetic antioxidants used to protect food products from lipid oxidation (Hamid *et al.,* 2015). However, these synthetic antioxidants may be hazardous to the consumer, which has led to increased demand for natural antioxidant (Halim *et al.,* 2016). There have been many studies on hydrolysates with antioxidant properties, such as those in eel (Halim and Sarbon, 2017) and scallops and abalone muscle (Zhou *et al.,* 2012). Therefore, there was a great interest in finding new and safe natural antioxidant compounds such as fish proteins hydrolyzed peptides (Jemil *et al.,* 2014).

Protein hydrolysates are breakdown products from protein compounds to smaller peptides which contain 2-20 amino acids by enzymatic conversion (Hamid *et al.,* 2015). However, bioactive peptides only function when released from native protein (Ranamukhaarachchi *et al.,* 2013). Generally, bioactive peptides can be obtained from fish proteins via enzymatic hydrolysis (Ishak and Sarbon, 2017). The proteolytic enzymes most commonly used to hydrolyze fish proteins for the production of fish protein hydrolysates are usually sourced from plants (papain, bromelain), animals (pepsin, trypsin, α-chymotrypsin), and microbes (Alcalase®, protamex, flavourzyme, neutrase) (Putra *et al.,* 2018). Due to its thermostability, enzyme Alcalase® is a highly efficient bacterial protease used for hydrolyzing muscle proteins for preparing protein hydrolysate (Hamid *et al.,* 2015).

Ultrafiltration (UF) is a membrane technology used in diverse fields such as food, pharmaceutical, and biotechnological industries for the concentration, purification and fractionation of various products (Foh *et al.,* 2010). The antioxidant activities of hydrolyzed protein were influenced by its molecular weight. This has been supported by work from Razali *et al.* (2015), who found that the low molecular weight of cobia skin gelatin hydrolysate (<3 kDa) is thought to have stronger DPPH radical scavenging activity.

In addition, the peptides and free amino acids produced via the enzymatic hydrolysis of native proteins will determine the changes in functional properties that may occur (Halim *et al.,* 2016). The functional quality of the hydrolysates produced commonly measured by properties such as solubility, emulsifying properties, foaming properties, and water holding capacity. The modification and improvement of functional characteristics of controlled enzymatic hydrolysis of protein result in the production of a series of smaller

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polypeptides for different applications (Jamil *et al.,* 2016).

*Ampulariidae*, commonly known as the apple snail, is a large family of freshwater snails. It typically consumes 7 to 24 rice seedlings per day of the young stem and leaves of paddies, causing extreme damage to growing paddies (Salleh *et al.,* 2012). The Golden apple snail is a major rice pest in Asia. It has been reported that these snails were initially introduced in Keningau, Sabah, Malaysia. However, the invasion of snail was spread to Peninsular Malaysia resulted in extensive damage to the young rice plants especially in Perlis and Kedah (Salleh *et al.,* 2012). In a previous study, optimization of the production of golden apple snail hydrolysate using Alcalase® was successfully conducted by Hamid *et al.* (2015). The hydrolysate produced showed high in antioxidant activity, chelating effects and hydroxyl scavenging activity. Therefore, this study aims to investigate the antioxidant activity and functional properties of fractionated protein hydrolysate from golden apple snails at three different molecular weight fractions (3, 5, 10 kDa).

## 2. Materials and methods

### *2.1 Materials*

Golden apple snails (*Ampullariidae*) (GAS) were obtained from Pekan, Pahang, Malaysia and were brought to the laboratory alive. The flesh was separated manually and thoroughly washed before mincing using Waring Blender (model HGB2WTS3). Then, the minced GAS was stored at -80$^{o}$C for further analysis. The enzyme Alcalase® 2.4L FG used was purchased from Novo Industry (Denmark). All chemicals used in this study were of analytical grade.

### *2.2 Preparation and fractionation of golden apple snail (Ampullariidae) hydrolysate (GASH) by ultrafiltration*

Hydrolysate of Golden apple snail (GASH) was prepared following the method as described by Hamid *et al.* (2015) and Razali *et al.* (2015) with some modification. Approximately 22±1 g of minced GAS flesh was mixed with 66 g distilled water. The GAS solution mixture was heated at 80$^{o}$C (15 mins) to inactivate endogenous enzyme that presents in the golden apple snail flesh. About 20 g of diluted Alcalase® enzyme at an enzyme-substrate concentration of 2% (prepared by diluting the required enzyme mass to a final weight of 20 g with distilled water) was added into the solution mixture. The GAS solution mixture was heated again in a water bath shaker at 45$^{o}$C and adjusting the pH using 4 N NaOH. The pH of the solution was kept constant at pH 6.7 while continuously stirring for 159 mins. In order to inactivate the Alcalase® enzyme activity, the hydrolysis was terminated by heating the solution at 90$^{o}$C for 15 mins. The resultant solution was centrifuged (Cryozen 1580R, Korea) at 5000 rpm (4$^{o}$C) for 20 mins. The supernatant obtained was then collected and fractionated to 3, 5 and 10 kDa using vivaspin ultrafiltration membrane (Vivaspin 20, Sartorius Stedim Biotech, Goettingen, Germany). Firstly, the GASH solution was allowed to pass through 10 kDa molecular weight cut-off (MWCO) membrane. Then, the filtrate obtained was consecutively filtered using 5 kDa and 3 kDa MWCO membranes. The obtained GASH fractions (<3, <5 and <10 kDa) were then freeze-dried and kept at -80$^{o}$C for further analysis.

### *2.3 Determination of antioxidative activities*

#### *2.3.1 Reducing power*

Reducing power of GASH was measured according to the procedure described by Hamid *et al.* (2015). Hydrolysate at 1.0 mg/mL for 1.25 mL (diluted with distilled water) of various molecular weights (3, 5, and 10 kDa) was mixed with phosphate buffer (1.25 mL) (0.2M, pH 6.6) and potassium ferricyanide (1.25 mL) (1%, w/v). The solution mixtures were then subjected to incubation at 50$^{o}$C (30 mins) before 1.25 mL of trichloroacetic acid (10%, w/v) were added. The mixture was then subjected to centrifugation (Cryozen 1580R, GRYOZEN Co. Ltm, the Yuseong, Daejon, Korea) at 10,000 rpm (10 mins). Then, the supernatant of solution (1.25 mL) was collected and were mixed with distilled water (1.25 mL) and 0.1% (w/v) Ferric Chloride (2.50 mL). Following incubation for 20 mins at room temperature, the solution absorbance was measured at 700 nm. BHT as synthetic antioxidant was used as positive control. A higher absorbance value was understood as indicating higher reducing power ability of the hydrolysate. Each test sample was examined in triplicate for each molecular weight.

#### *2.3.2 DPPH radical scavenging activities*

The DPPH radical-scavenging assay was used to investigate the ability of compounds to act as free radical scavengers or hydrogen donors per the methods used by Razali *et al.* (2015). Briefly, a volume of 500 µL of each hydrolysate with different molecular weight (3, 5, and 10 kDa) was mixed with ethanol (500 µL) and DPPH in 99.5% ethanol (125 µL) at 0.02%, (w/v). The solution mixture was vortexed before subjected to incubation in a dark place at room temperature. The absorbance of the supernatant was measured via UV-Visible spectrophotometer at 517 nm (Spectroquant® Pharo 300, Darmstadt, European Union) after 60 mins incubation. The DPPH-radical scavenging activity was calculated as follows:

 

DPPH radical scavenging activity (%) = [($A_0$ – $A_1$)/ $A_0$] x 100

Where $A_0$ = The absorbance of the control, and $A_1$ = The absorbance of the mixture containing sample.

The synthetic antioxidant reagent Butylated Hydroxytoluene (BHT) was used as a positive control. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. The tests were conducted in triplicate.

*2.3.3 Metal chelating activity*

Metal chelating activity on ferrous ion of different molecular weight (3, 5, and 10 kDa) of GASH fraction was determined following Hamid *et al.* (2015) with slight modification. Aliquot solution of GAS hydrolysate (500 µL) at a concentration of 1 mg/mL was mixed with $FeCl_2$ (0.05 mL). The addition of 5mM ferrozine (0.1 mL) was initiated the reaction to occur. Then after shaken vigorously, the mixture was left to stand at room temperature for 10 min. The resulting solution was measured at an absorbance of 562 nm using a spectrophotometer (Spetrouant Pharo 300, Europe). The chelating antioxidant activity was calculated as follows:

Metal chelating effect (%) = [($A_0$ – $A_1$)/ $A_0$] X 100

Where $A_0$ = The absorbance of the control, and $A_1$ = The absorbance of the mixture containing sample

The positive control (Butylated hydroxytoluene, BHT) was prepared in the same manner, except that distilled water was used instead of a trial sample. The tests were conducted in triplicate.

*2.4 Determination of functional properties*

*2.4.1 Emulsifying properties*

The emulsifying properties were measured according to the procedure of Razali *et al.* (2015). The emulsions were prepared by homogenizing 45mL GASH solution with pure vegetable oil (Vsawit) (15 mL) using a mechanical homogenizer (Ultraturrax T18) at 16 000 rpm (1 min). After homogenization, samples were taken (500 µL) at 0 min and 10 mins from the bottom of the container before diluted with 10 mL SDS solution (0.1%). Diluted emulsion formation at 0 min and 10 mins was then measured at 500 nm. The emulsifying activity index (EAI) and emulsifying stability index (ESI) of each emulsion were calculated as follows:

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times protein\ weight\ (g)}$$

$$ESI\ (\text{min}) = \frac{A_0 \times \Delta t}{\Delta A}$$

Where $\Delta A = A_0 - A_{10}$, and $\Delta t$= 10 mins

The test was conducted in triplicate.

*2.4.2 Foaming properties*

Foaming capacity was measured using the method described by Shahidi *et al.* (1995). Each of GASH fraction (1 g) was added with distilled water (50 mL). The solution mixture was then homogenized (16,000 rpm) for 1 min by using Ultraturrax T18 homogenizer. The height of solution was measured at 0, 0.5, 1, 5, 10, 20, and 30 min after whipping, respectively and the foaming capacity index (%) was calculated as follows:

$$Foaming\ Capacity\ Index\ (\%) = \frac{Volume\ after\ whipping\ (mL)}{Volume\ before\ whipping\ (mL)} \times 100$$

Measurements were conducted in triplicate.

*2.4.3 Solubility*

Protein solubility was measured according to the method described by Castro and Sato (2014). About 100 mg of GASH was dispersed in 10 mL of distilled water (pH 6.5±0.3) at room temperature. Then, the mixture was centrifuged (Cryozen 1580R, GRYOZEN Co. Ltm, the Yuseong, Daejon, Korea) at 17,000 x *g* for 10 mins. The total protein content of the GASH was determined using Kjedahl method (AOAC, 2002), while the protein content in the supernatant was determined using the Biuret method. Protein solubility was calculated using the following expression:

$$Solubility\ (\%) = \frac{Protein\ content\ in\ supernatant\ (g)}{Protein\ content\ in\ GASH} \times 100$$

*2.4.4 Water binding capacity*

Water binding capacity was determined using the method described by Razali *et al.* (2015). Each GASH fraction (0.5 g) was dissolved in distilled water (10 mL) in centrifuged tube followed by vortexing for 30 s. After that, the solution mixture was allowed to stand at room temperature for 30 mins before being centrifuged at 4840 rpm for 25 mins (Cryozen 1580R, GRYOZEN Co. Ltm, the Yuseong, Daejon, Korea). The mixture was filtered by using Whatman No. 1 and the volume recovered was measured. The water binding capacity of GASH was calculated as follows:

$$Water\ Binding\ Capacity\ (\%) = \frac{Volume\ of\ water\ (mL)}{Weight\ of\ GASH\ (g)} \times 100$$

Measurements were conducted in triplicate.

*2.6 Statistical analysis*

All the tests were done in triplicate and the data were averaged. All data obtained was subjected to one-way analysis of variance (ANOVA) and stated as mean ± standard deviation. MINITAB Statistical Software 14.0 and Microsoft Excel were used for all analyses and the

 

Fisher LSD test was used to evaluate significant differences ($p<0.05$) between the means for each sample.

## 3. Results and discussion

### *3.1 Yield of Golden apple snail hydrolysate (GASH)*

Table 1 shows the yield obtained for the Golden apple snail hydrolysate (GASH) and its fractions (3, 5, and 10 kDa). The yield of unfractionated GASH was 12.62%, which was higher than that of a study conducted by Hamid *et al.* (2015), (9.72%). While, the fractionate GASH yielding 1.45%, 3.67% and 3.34%, respectively for fraction 3, 5, and 10 kDa. The lowest yield at low molecular weight was because the peptides formed during protein hydrolysis reaction were themselves degraded into smaller peptides. The same trends were reported by Razali *et al.* (2015) for cobia skin gelatin hydrolysate. However, this finding contrasts with a study conducted on rapeseed hydrolysate which reported the yield increased with decreasing molecular weight fractions (He *et al.,* 2012). The differences in yield obtained depended on several factors such as pre-treatment, extraction time, temperature, pH, and peptide chains during the hydrolysis process.

Table 1. The yield of Golden apple snail hydrolysate and its fractions at different molecular weights (3, 5, 10 kDa).

| Hydrolysate | Yield (%) |
|---|---|
| Unfractionated GASH | 12.62±0.74 |
| 3kDa | 1.45±1.25$^{b}$ |
| 5kDa | 3.67±0.92$^{a}$ |
| 10kDa | 3.34±1.82$^{a}$ |

All results expressed as the mean (± SD) of three trials. Different superscript letters indicate significant differences between means ($p<0.05$).

### *3.2 Antioxidative properties*

#### *3.2.1 Reducing power*

The reducing power of the GASH fractions was measured by Potassium ferricyanide. Higher reducing power of the sample was indicated by higher absorbance obtained. The reducing power of different Golden apple snail hydrolysate (GASH) fraction (3, 5, and 10 kDa) and BHT that acts as a positive control at a concentration of 1 mg/mL was shown in Table 2. All GASH fractions (3, 5, and 10 kDa) were significantly different ($p<0.05$) as compared to the positive control, BHT. Based on the results obtained, 3 kDa shows the highest ($p<0.05$) reducing power compared to 5 kDa and 10 kDa. However, BHT demonstrated the highest reducing power for all tested samples at 1.75 nm. The higher reducing power obtained by 3 kDa was due to lower molecular weight fractions having higher hydrophobic amino acid levels compared to the high molecular weight fraction that more easily to donate an electron and hydrogen atom in order to stabilize the $Fe^{2+}$. A similar finding was also obtained by Saidi *et al.* (2014). The findings show that a low molecular weight of hydrolysate (1-4 kDa) has better reducing power than those of higher molecular weight (>4 kDa). The highest reducing power increase with decreasing peptide length or molecular weight indicates that higher electron reduction potential resulted from lower peptide chains (Razali *et al.,* 2015). Compounding with reducing power indicates an electron donor and can reduce the oxidized intermediates of lipids peroxidation process that makes the compound develop into primary and secondary antioxidants (Jayanthi and Lalitha, 2011).

#### *3.2.2 DPPH radical scavenging activity*

Table 2 shows the DPPH radical scavenging activity of different Golden apple snail hydrolysate (GASH) fractions (3, 5, and 10 kDa) with BHT as the positive control. There were significant differences in DPPH radical scavenging activity for all GASH fractions (3, 5, and 10 kDa) as compared to the positive control (BHT) ($p<0.05$). The radical scavenging activity of 3 kDa fraction (79.10%) was higher compared to BHT, 5 kDa and 10 kDa. Fraction 3 kDa showed higher DPPH radical scavenging activity compared to other fractions, perhaps due to its low molecular weight of peptides. Low molecular peptides have more exposure to the residue side chain and are responsible for facilitating the reaction between peptides and free radicals, thus increasing DPPH radical scavenging activity (Zhou *et al.,* 2012). These findings were in agreement with a study conducted by Razali *et al.* (2015), in which scavenging activity of GASH was able to stabilize the DPPH radical by acting as a proton donating substance.

Table 2. Antioxidant activity of Golden apple snail hydrolysate and its fractions at different molecular weight (3, 5, 10 kDa).

| Hydrolysate | Antioxidant activity | | |
|---|---|---|---|
| | Reducing power (nm) | DPPH radical scavenging (%) | Metal chelating (%) |
| BHT | 1.75±0.03$^{a}$ | 66.35±1.43$^{b}$ | 90.16±0.07$^{ab}$ |
| 3kDa | 0.90±0.03$^{b}$ | 79.10±0.30$^{a}$ | 80.86±0.87$^{bc}$ |
| 5kDa | 0.82±0.02$^{bc}$ | 18.37±0.06$^{cd}$ | 85.60±0.71$^{a}$ |
| 10kDa | 0.72±0.02$^{d}$ | 20.42±0.69$^{c}$ | 72.84±3.49$^{cd}$ |

All results expressed as the mean (± SD) of three trials. Different superscript letters indicate significant differences between means ($p<0.05$).

 

*3.2.3 Metal chelating activity*

The metal chelating activity on fractionated Golden apple snail hydrolysate (3, 5, and 10 kDa) at 1mg/mL was shown in Table 2. These findings indicate that metal chelating activity was not affected significantly ($p>0.05$) by 3 and 5 kDa from BHT. GASH exhibited the highest metal chelating activity (85.60%) at 5 kDa, yet the result still lower than BHT value (90.16%) that acts as the positive control. Higher metal chelating activity was probably due to the increasing carboxylic group ($COO^-$) and amino group in branches of the acidic and basic amino acids with low molecular weight, thus enhancing the binding of $Fe^{2+}$. These results are in agreement with research conducted by Taheri *et al.* (2014), with herring brine, as 10-1 kDa molecular weight of herring brine has higher chelating activity compared to molecular fraction >50 kDa. Taheri *et al.* (2014) also stated that low molecular fraction 10-1 kDa is the best metal chelator. Iron is one of the common initiators of lipid peroxidation, resulting in the acceleration of lipid decomposition (Liu *et al.,* 2012). Ferrozine can quantitatively form complexes with $Fe^{2+}$. In the presence of chelating agents, this formation is disrupted, resulting in a decrease in the red color of the complex (Zhu *et al.,* 2006). Metal chelating capacity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. Thus, the chelating agent can decrease the redox potential and therefore stabilize the oxidized form of the metal ion.

*3.3 Functional properties*

*3.3.1 Emulsifying properties*

Figure 1 (a) shows the emulsion activity index (EAI) in different Golden apple snail hydrolysate (GASH) fraction. Results indicated that EAI value was affected significantly ($p<0.05$) with the different fraction of GASH. Emulsion activity index (EAI) decreased as fractional molecular weight increased and the hydrolysis process appeared to improve GASH EAI for 3 kDa to 10 kDa fractions. A study reported by Razali *et al.* (2015) also indicated that increasing EAI values led to decreasing molecular weight fractions. The higher surface hydrophobicity was obtained by 3 kDa fraction. The protein structure and function alteration of the hydrolysate via partial hydrolysis may facilitate diffusion at the oil/water interface which resulted from higher solubility and smaller molecular size (Wu *et al.,* 1998). In addition, partial hydrolysis could lead to the unfolding of protein molecules. Nonpolar amino acid groups buried inside protein molecules could be exposed on the surface of protein molecules after unfolding (Miroljub *et al.,* 2006). Thus, the enhanced interactions between proteins and lipids would be obtained by hydrophobic groups exposed (Razali *et al.,* 2015).

In addition, the emulsion stability indexes (ESI) for all three GASH fractions are shown in Figure 1b. It was observed that 3 kDa was significantly affected ($p<0.05$) with 5 and 10 kDa fractions. In contrast with EAI value, the ESI for all samples decreased (106.87%, 30.35%, 23.71%) as the molecular weight of the hydrolysate fraction decreased (10, 5, 3 kDa). Similar results were obtained by Razali *et al.* (2015) on cobia skin gelatin hydrolysate (CSGH). The higher ESI for large molecular peptides compared to low molecular peptides is due to small peptides having short chain of amino acid that lead to the lack of unfolding at the interface and have low efficiency in reducing the interfacial tension compared to the large molecular peptide (Gbogouri *et al.,* 2004). In order to exhibit better emulsifying activity, proteins and/ or peptides must migrate rapidly to the water/oil interface where they quickly unfold and rearrange the interface (Kotlar *et al.,* 2013). The hydrophobic/ hydrophilic balance of smaller peptides is insufficient for the stabilization of emulsions, although peptides with lower molecular weights can migrate rapidly to the interface (Deng *et al.,* 2011). Hence, due to a reduction in the hydrophobic/hydrophilic balance, smaller hydrolysate fractions result in decreasing emulsion stability.

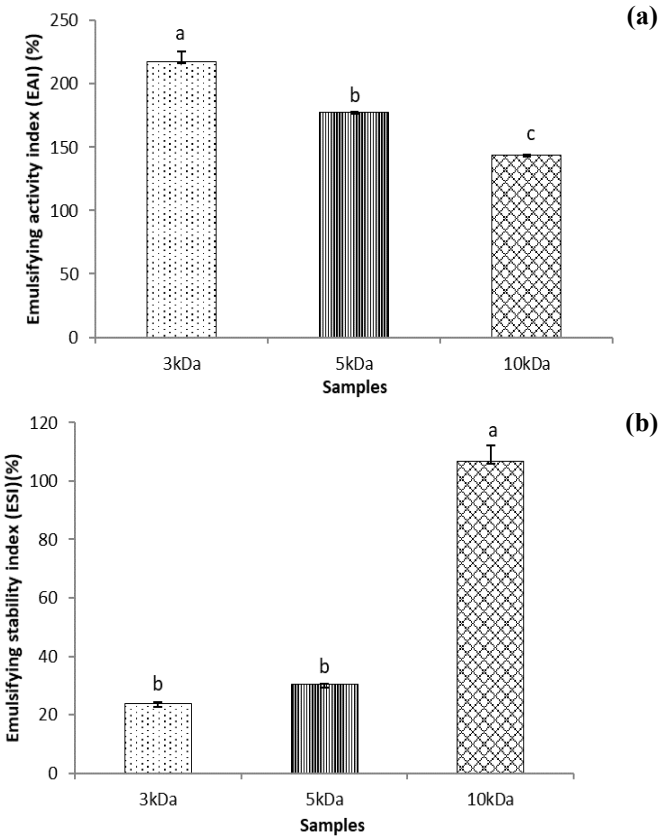


Figure 1. The (**a**) Emulsifying activity index (EAI) (**b**) Emulsifying stability index (ESI) at different molecular fraction (3, 5, and 10 kDa) of Golden apple snail hydrolysate (GASH) at concentration of 1 mg/mL. Results presented as mean ± SD. The different letters indicate there is significant difference ($p<0.05$) between samples.

### 3.3.2 Foaming capacity

Figure 2 (a) shows the foaming capacity for all GASH fractions. The results showed that the foaming capacity of all molecular weights (3, 5, and 10 kDa) decreased over time. However, there was no significant difference in foaming capacity between these molecular weights in terms of time ($p>0.05$). Among all molecular weights (3, 5, and 10 kDa), fraction 5 kDa had the highest value compared to 3 kDa and 10 kDa, at 50.0, 5.66 and 40.0%, respectively. The decrease in peptide molecular weight leads to the decreased in foaming capacity because small peptides do not have the ability to stabilize the air cells of the foam. Furthermore, smaller peptides allow quick absorption of water thus lowering the surface tension. Similar results were obtained by Barac *et al.* (2011). In smaller molecular peptide fractions, protein hydrolysate is unable to form a strong bond at air/water interface thus experienced more gravitational pull than unmodified isolates. However, research conducted by Razali *et al.* (2015) on cobia skin gelatin hydrolysate showed different results from the findings in GASH fraction, as 5 kDa obtained the highest foaming capacity, followed by 10 kDa and 3 kDa. This may be due to the different protein-protein interaction of the fraction in the surface of water and air, thus affecting the foaming capacity of the protein hydrolysate at the different molecular weight. Cohesive interactions between molecules at air-liquid interfaces lead to the formation of surface tension. Thus, substances with amphiphilic properties can adsorb at air-liquid interfaces, decreasing the surface tension and resulting in foams with increased kinetic stability (Patino *et al.,* 2008).

### 3.3.3 Solubility

Figure 2 (b) shows the solubility index of different Golden apple snail hydrolysate fraction (3, 5, and 10 kDa). There were significant differences ($p<0.05$) in solubility index for all fractions and 5 kDa had the highest solubility index compared to 3 kDa and 10 kDa. This might be due to the presence of more hydrophilic amino acid exposed to the water, leading to the formation of hydrogen bonds with water and increasing its solubility index. According to Wu *et al.* (1998), when amino acids are exposed, they will interact with water molecules through hydrogen bonds and electrostatic interaction and thus solubilize the hydrolysate. These findings were slightly different from a previous study conducted by Wang *et al.* (2006) on wheat gluten, in which the lowest molecular weight 20 kDa shows the highest solubility index compared to the other fractions, i.e. 50 kDa and 100 kDa in various range of pH. Smaller peptide has shorter peptide length and have fewer hydrophobic binding sites but more hydrophilic group that exposed to the water surrounding thus increasing its solubility (Wu *et al.,* 1998). In addition, the exposure of more charged and polar groups of low molecular weight to the surrounding water increased the solubility of low molecular peptide fraction (Jemil *et al.,* 2014). Thus, molecular fractions were shown to have an effect on the solubility index of protein hydrolysate in water. GASH with high solubility over a wide pH range can, therefore, be widely applied in formulated food systems.

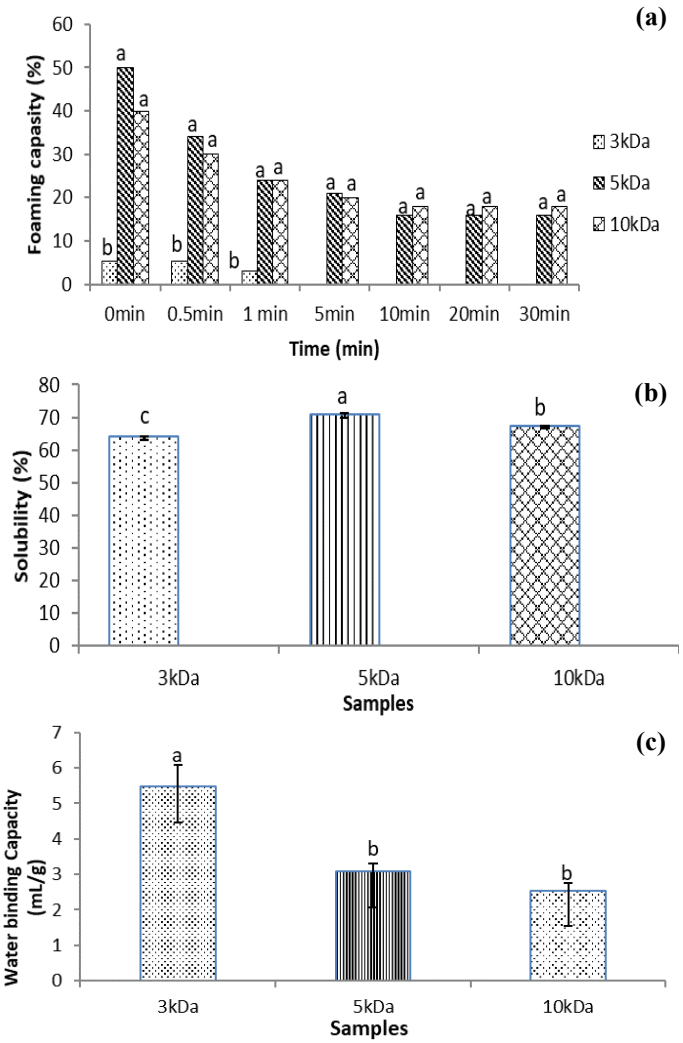


Figure 2. The (**a**) foaming capacity (**b**) solubility (**c**) water binding capacity (WBC) at different molecular fraction (3, 5, and 10 kDa) of Golden apple snail hydrolysate (GASH) at concentration of 1 mg/mL. Results presented as mean ± SD. The different letter indicates a significant difference ($p<0.05$) between samples.

### 3.3.4 Water binding capacity

The water binding capacity (WBC) of fractionate Golden apple snail hydrolysate (GASH) at the different molecular weight (3, 5, and 10 kDa) were shown in Figure 2 (c). This finding indicates that fraction (3 kDa) had higher water binding capacity compared to fraction 5 kDa and 10 kDa which were 5.47, 3.07 and 2.53 mL/g, respectively. However, there was no significant difference ($p<0.05$) on WBC between fraction 5 kDa and

 

10 kDa. The greater water binding capacity of 3 kDa may be due to enzymatic hydrolysis, resulting in fewer peptide fragments, as smaller peptides have higher hydrophilic functional groups. Furthermore, low molecular weight peptide fragments are more effective in holding water than larger peptide sizes (Cumby *et al.,* 2007). These results are similar to previous findings on cobia skin gelatin hydrolysate as reported by Razali *et al.* (2015). They show that the 3 kDa and 5 kDa peptide fractions had the highest water binding capacity compared to 10 kDa. Therefore, GASH may act as a good water binder for food products.

## 4. Conclusion

In conclusion, protein hydrolysate from golden apple snail (GAS) with different molecular weight (3, 5, and 10 kDa) affects the yields, antioxidant activity and also the functional properties of hydrolysate produced. The results indicate that golden apple snail hydrolysates (GASH) with different molecular weights have potent antioxidant activity and good functional properties. The functional properties of GASH were significantly affected by the size of molecular weight. In other words, fraction of <3 kDa may have good antioxidant activity but not necessarily it will be good in functional properties. Thus, antioxidant and functional properties are significantly affected by the molecular weight of hydrolysate.

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