

Effects of Enzyme Concentration, Temperature, pH and Time on the Degree of Hydrolysis of Protein Extract from Viscera of Tuna (*Euthynnus affinis*) by Using Alcalase

(Kesan Kepekatan Enzim, Suhu, pH dan Masa ke atas Darjah Hidrolisis Ekstrak Protein daripada Visera Tuna (*Euthynnus affinis*) Menggunakan Alcalase)

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ABSTRACT

Protein from viscera of tuna was extracted by using water at ratio of viscera to water of 1:1 (w/v) and freeze-dried. The protein was found to be high in glycine (9.6%), arginine (9.2%), alanine (7.0%), lysine (7.2%) and leucine (7.0%). A study was then carried out to determine the effect of alcalase concentration, temperature, pH and incubation time on degree of hydrolysis (DH) during hydrolysis by using single factor experiment. The hydrolysis of viscera protein extract (VPE) was carried out at concentrations of 1.0, 1.5 and 2.0%. The DH of hydrolysates was significantly ($p < 0.05$) increased when enzyme concentration was increased from 1.0% to 1.5% but became constant at concentration exceeding 1.5%. An enzyme concentration at 1.5% was subsequently used in the study of the effect of the other parameters. It was found that the value of the DH also increased when the temperature was increased from 30 to 40°C. However, the hydrolysis at higher temperature (60°C) produced lower DH. Prolonging the time of incubation from 60 min up to 240 min significantly ($p < 0.05$) increased the DH. As for pH, there were no significant effects observed.

Keywords: Alcalase; hydrolysis; tuna; viscera

ABSTRAK

Protein daripada bahan buangan tuna diekstrak menggunakan air pada nisbah 1:1 (w/v) dan dikering sejuk bekuan. Ekstrak protein adalah tinggi dengan kandungan glisin (9.6%), arginin (9.2%), alanin (7.0%), lisin (7.2%) dan leusin (7.0%). Suatu kajian seterusnya dijalankan untuk menentukan kesan kepekatan enzim alkalase, suhu, pH dan masa eraman ke atas darjah hidrolisis (DH) semasa hidrolisis menggunakan eksperimen faktor tunggal. Ekstrak protein visera dilakukan pada kepekatan 1.0, 1.5 dan 2.0%. DH bagi hidrolisat meningkat dengan signifikan pada $p < 0.05$ apabila kepekatan enzim meningkat daripada 1.0 ke 1.5% tetapi menjadi malar pada kepekatan melebihi 1.5%. Kepekatan enzim pada 1.5% digunakan dalam kajian seterusnya untuk menentukan parameter-parameter lain. Didapati nilai DH juga bertambah dengan signifikan ($p < 0.05$) apabila suhu ditingkatkan daripada 30-40°C. Walau bagaimanapun, hidrolisis pada suhu lebih tinggi (60°C) menghasilkan DH yang lebih rendah. Pemanjangan masa eraman daripada 60 ke 240 min, meningkatkan DH secara signifikan ($p < 0.05$). Bagi pH pula, tiada kesan yang signifikan ke atas nilai DH diperhatikan.

Kata kunci: Alcalase; hidrolisis; tuna; visera

INTRODUCTION

Tuna fisheries are a high potential industry, which contributes high income to the country. In 2006, tuna was the third most important fisheries product traded. Under the National Agricultural Policy, Balance of Trade on Food Sector Plan and since the Ninth Malaysian Plan, exploitation of tuna had become one of the main agenda whereby tuna is targeted to be the main contributor of fish production and contribute to Malaysia's endeavor to be among the net exporter of food (Noraisyah & Raja Bidin 2011).

Malaysia has imported about RM 908 million and at the same time has exported RM 1,232 million worth of fish and fish products. With respect to tuna, total imports registered were RM 53.4 million while exports RM 52.7 million

(Tuna Investment 2001). Malaysia's tuna productions are generally stable where the annual trend of tuna landings in Malaysia between 2000 and 2009 showed an increase from 59337 to 67221 tonnes. There was a slight decrease in 2009 (56480 tonnes) from previous landing in 2008 but then increased again in 2010. From these amounts, more than 90% of the tuna landing in Malaysia comprises of the species *Euthynnus affinis* and *Thunnus tonggol* (Noraisyah & Raja Bidin 2011).

Euthynnus affinis is one of the neritic tuna species that could be found in the Malaysian waters and has been listed as highly migrated species and become a dominant species starting from 1992. Most of the tuna caught are consumed fresh where fish consumption is relatively high in Malaysia. Tuna products which are consistently

in demand in Malaysia includes fresh/chilled and frozen fish as well as for preserved fish (Tuna Investment 2001). This species also come with low-mercury and a less expensive substitute for canned albacore since 2004 as noted by USFDA resulted from the occurrence of natural accumulation of heavy metals of tuna up the food chain. As a result, a large amount of by-product materials were disposed annually from tuna processing industry. These raw materials or wastes, which, if not utilized may cause environmental, health and economical problems. Hence, optimal utilisation of the raw material is of prime importance to cope for the increasing demand for marine oils and proteins (Arnesen & Gildberg 2006).

The fish by-products mainly serve as a cheap ingredient in animal feed production (Arnesen & Gildberg 2006). In order to increase the utilization of these fishery waste products, processes such as enzymatic hydrolysis of the fish proteins to produce a more marketable and functional protein hydrolysate should be considered (Aspmo et al. 2005; Liceaga-Gesualdo & Li-Chan 1999). The functional properties of fish protein hydrolysates prepared may be improved through partial hydrolysis where the proteins are hydrolyzed to the desired extent by the use of specific enzymes and by choosing a defined set of hydrolysis conditions. Parameters such as time, pH, temperature and enzyme concentration influence enzymatic activity co-operatively. Hence this offer possibilities to control the process (Hall & Ahmad 1992; Liaset et al. 2000; Viera et al. 1995).

There are a number of different proteolytic enzymes that can be used for the production of hydrolysates (Liceaga-Gesualdo & Li-Chan 1999). Proteolytic enzymes from plants and microorganisms have been found to be more suitable to produce fish protein hydrolysate (Bhaskar et al. 2008). According to Mullaly et al. (1995), enzymes used to produce fish protein hydrolysate should be either a food grade enzymes or non-pathogenic if it is from a microbial origin.

Alcalase is a commercially available enzyme preparation that has been widely used in the production of such protein hydrolysates because of its thermostability (50°C) and high optimal pH (pH8.5), which can minimize the growth of microorganisms during hydrolysis process. Produced from a selected strain of *Bacillus licheniformis*, the main enzyme component, subtilisin A (generic name, Subtilisin Carlsberg), is an endopeptidase; it is available as a food grade preparation that complies with the FAO/WHO JECFA and FCC (Novo Nordisk 1995). Another advantage of using alcalase is that it provides extensive proteolysis with less bitterness of protein hydrolysates compared with others (Liceaga-Gesualdo & Li-Chan 1999).

The application of alcalase to produce protein hydrolysate from other source of fishes and seafood by-products has been previously reported (Bhaskar et al. 2007; Hoyle & Merritt 1994; Sathivel et al. 2005). However, to the authors' best knowledge, there are no studies done on the visceral waste of tuna fish (*Euthynnus affinis*). Therefore, this study was carried out to determine the composition of tuna viscera to see its potential as a

good source of protein which later can be converted into a more functional protein hydrolysate. Furthermore, we wish to study the effects of the critical parameters which are pH, enzyme concentration, temperature and hydrolysis time on the degree of hydrolysis of protein hydrolysate produced.

MATERIAL AND METHODS

PREPARATION OF PROTEIN EXTRACT

Tuna fish bought from the local market in Kajang were eviscerated. The protein extract was prepared according to the report by Bhaskar et al. (2008) with modification on the centrifuge speed, time and temperature as to allow the samples to have an optimum separation. The visceral were washed with water for several times to remove some of the blood. It was then minced by using Waring blender followed by heating the visceral mass at 85°C for 20 min to aid in inactivating the endogenous enzyme and to facilitate the release of the fat from the mass. The heat treated visceral mass was allowed to cool and centrifuged at 4°C for 20 min at 12000 g to separate the oil. The separated oil were removed and the protein rich solids were then collected and extracted thrice with distilled water at 1:1 (w/v) to get the protein extract. This protein extract (PE) was then freeze dried and used in the preparation of the protein hydrolysate. Proximate composition (protein, fat, moisture, ash and carbohydrate) of the raw material and PE were determined according to the AOAC method (AOAC 1990).

PREPARATION OF PROTEIN HYDROLYSATE

Hydrolysis of protein was done by enzymatic hydrolysis using alcalase, an alkaline bacterial protease as a biocatalyst. Approximately 6 g of freeze dried PE were dissolved in 100 mL of phosphate buffers (0.1 M, pH7, 8 and 9) and heated at 95°C for 15 min. The hydrolysis was carried out by mixing 50 mL of protein solutions with 50 mL enzyme solutions of different concentrations to give the final enzyme concentration of 1.0%, 1.5% and 2.0% (w/w substrate). The mixture was then incubated at a speed of 200 rpm in a shaker incubator (Environ-Shaker) for 60, 120 and 240 min at 30°C, 40°C and 60°C. The reaction was stopped by heating the mixture at 95°C for 15 min to deactivate the enzyme. The samples were then centrifuged at 2600 × g for 15 min in order to separate the enzyme and impurities from the hydrolysate produced. The supernatant (protein hydrolysate) was collected and the degree of hydrolysis was analyzed before freeze dried. A one factor experimental design was used throughout the studies where only one parameter was varied at each hydrolysis.

DEGREE OF HYDROLYSIS (DH) DETERMINATION

The DH was measured according to the method reported by Hoyle and Merritt (1994). At the end of each reaction, an aliquot (10 mL) was removed, mixed with 10 mL of

20% trichloroacetic acid (TCA) to obtain 10% TCA-soluble nitrogen and then centrifuged at $2600 \times g$ for 15 min. The supernatant was decanted and analyzed for nitrogen content by Kjeldahl method (AOAC 1990) using Kjeltex protein analyzer (FOSS-Tecator 2000). The degree of hydrolysis was calculated as:

$$\% \text{ DH} = \frac{\text{Soluble N in 10\% TCA}}{\text{Total N in the sample}} \times 100,$$

where, N is nitrogen and TCA is trichloroacetic acid

DETERMINATION OF NITROGEN CONTENT

Nitrogen content was determined by Kjeldahl standard method using protein analyzer (KjeltexTM 2000, Foss-Tecator, Sweden) according to AOAC (1990) method. Nitrogen content was calculated by the formula below:

$$\% \text{ N} = \frac{0.1 \times \text{Titration volume (mL)} \times 14 \times 10}{\text{Sample weight (g) or volume (mL)} \times 1000},$$

where N is nitrogen.

AMINO ACID ANALYSIS

The determination of amino acid compositions was done according to Blackburn (1986) and AOAC (2000) where the samples were hydrolysed by using 6N HCl (amino acids other than cysteine, methionine and tryptophan), LiOH 4.3N (tryptophan) and performic acid (cysteine and methionine). Then, the free amino acids were separated on a HPLC using a (3.9 mm \times 150 mm) waters accQ.tag amino acid analysis column at 36°C. The identity and quantity of the amino acids were determined by comparison with the retention times and peak areas of each amino acid standard [acid L-alpha-amino-n-butirik (AABA)].

CHEMICAL SCORE

The chemical score of the protein hydrolysate was computed as per Ovissipour et al. (2009). It was calculated relative to the essential amino acid (EAA) profile in a standard protein as described by FAO/WHO (1990) or NRC (1993). In brief, the chemical score was calculated by using the following equation:

$$\text{Chemical score} = \frac{\text{EAA in sample protein (g/100 g)}}{\text{EAA in standard reference protein (g/100 g)}}.$$

The data were analysed using Statistical Analytical System (SAS) version 6.12 for ANOVA test and DUNCAN. All experiments were done in three replications.

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION OF TUNA VISCERA

As shown in Table 1, the tuna viscera contained 65.04% protein, 11.77% fat, 75.73% moisture, 3.12% ash and 17.54% carbohydrate. However, there were a significant ($p < 0.05$) increase of protein ($78.17 \pm 2.91\%$) and decrease of fat ($0.23 \pm 0.15\%$) in the protein extract. The differences were caused by the removal of fat and impurities during extraction as reported by Bhaskar et al. (2008). The reduced fat content in the extract will make it a good proteinaceous source for producing stable protein hydrolysate since the presence of protein-lipid complex will cause resistance to protein hydrolysis (Nilsang et al. 2005; Slizyte et al. 2005).

AMINO ACID COMPOSITION OF PROTEIN EXTRACT (PE)

Table 2 shows the contents of free amino acids in the tuna viscera and the protein extract (PE). The total of amino acid content of viscera and PE were 51.07% and 56.28% respectively. Glycine, arginine, alanine, lysine and leucine were the major amino acids present in both viscera and PE. Spurvey et al. (1998) reported that arginine is normally present in large amounts in lots of seafood. It contributes to a pleasant overall preference rather than bitterness to those products. Although glutamic acid and aspartic acid were among the higher amino acids detected, their amount might be overestimated since they can be formed from the deamination of glutamine and asparagines, respectively, during acid hydrolysis (Chen & Zhang 2007; Tyler 2000).

Previous research has found that the hydrophobic character of an amino acid plays the most significant role in creating the perception of bitterness (Chen & Zhang 2007; Ishibashi et al. 1988). The amino acids that are hydrophobic

TABLE 1. Proximate composition of tuna (*Euthynnus affinis*) viscera and protein extract (dry weight basis)

Proximate composition (%)	Viscera	Protein extract
Protein	65.04 ± 1.40^b	78.17 ± 2.91^a
Fat	11.77 ± 1.41^a	0.23 ± 0.15^b
Moisture	75.73 ± 0.33	-
Ash	3.12 ± 0.11	-
Carbohydrate	14.54 ± 7.50	-

**b Different letters within the same row denote significant differences ($p < 0.05$)

or aromatic and known to cause bitterness include valine, alanine, leucine, isoleucine, phenylalanine and tryptophan (Humiski & Alusko 2007). Additionally, the viscera and PE consisted of hydrophobic amino acids, such as leucine (3.5 to 5.0%), isoleucine (2.0 to 3.0%), phenylalanine (1.7 to 2.2%) and valine (2.6 to 2.9%). As for proline, the percentage is about 2.4-2.9%. Other than phenylalanine, another aromatic amino acids, tyrosine were present in small amounts in both viscera and PE and the sensitive amino acids, such as methionine and tryptophan, were also present in smaller amounts while cysteine was the most limiting amino acids in these material.

Bitterness was one of the main contributors to off-flavour of fish hydrolysate (Thiansilakul et al. 2007). Bitterness of protein hydrolysates is associated with the release of peptides containing hydrophobic amino acid residues (FitzGerald & O'Cuinn 2006). Thus the peptidase, which can cleave the hydrophobic amino acids and proline, is capable of producing a less bitter protein hydrolysate (Capiralla et al. 2002). Bhaskar et al. (2008) stated that alcalase, an alkaline bacterial protease produced from *Bacillus licheniformis*, has been proven to be the best enzyme in the preparation of fish protein hydrolysate. In addition, Kristinsson and Rasco (2000) reported that fish protein hydrolysates prepared using alcalase had less bitter characteristics. Alcalase has also been reported to be a better candidate for hydrolyzing fish proteins based

on enzyme cost per activity. Hence, alcalase was used in this study to produce the hydrolysate from the PE of tuna viscera.

HYDROLYSATES OF TUNA VISCERA

In this work, there were four different parameters studied which were enzyme concentrations, temperature, pH and time of incubation. Only one parameter was varied at one time as to see its effect alone not considering interactions with other parameters. James et al. (2005) reported that enzyme concentration generally had a greater effect on reducing hydrolysis time than temperature increment. Thus, the effect of alcalase on the DH of tuna viscera protein extract was initially done on enzyme concentrations of 1.0, 1.5 and 2.0%. At this point, temperature, pH and time of incubation were fixed at 40°C, pH8 and 60 min. Figure 1 shows that DH of tuna viscera increased as the alcalase concentration increases. DH value was significantly higher ($p<0.05$) at 1.5% alcalase concentration compared with 1.0%. The result obtained may be due to the increased of amino acids and smaller peptides present in the hydrolysate as some of the peptides released were greater hydrolyzed when the alcalase concentration increased. Increasing enzyme concentration to 1.5% allowed the occurrence of hydrolysis at a higher degree thus results in a higher DH. See et al. (2011) in their study of enzymatic hydrolysis of

TABLE 2. Comparison of amino acids composition between Tuna viscera and protein extract

Amino acid	Viscera (%)	Protein extract (%)
Aspartic acid	4.80 ± 0.49 ^a	5.16 ± 0.95 ^a
Serine	2.31 ± 0.17 ^a	2.61 ± 0.73 ^a
Glutamic acid	6.17 ± 0.34 ^a	7.10 ± 1.83 ^a
Glycine	4.89 ± 0.36 ^a	4.52 ± 1.12 ^a
Histidine	1.72 ± 0.12 ^a	2.40 ± 0.94 ^a
Arginine	4.72 ± 0.27 ^a	5.41 ± 1.37 ^a
Threonine	2.42 ± 0.17 ^a	2.70 ± 0.69 ^a
Alanine	3.58 ± 0.32 ^a	3.57 ± 0.78 ^a
Proline	2.64 ± 0.22 ^a	2.58 ± 0.48 ^a
Tyrosine	1.21 ± 0.06 ^a	1.62 ± 0.29 ^a
Valine	2.60 ± 0.10 ^a	2.87 ± 0.51 ^a
Lysine	3.71 ± 0.30 ^a	4.31 ± 0.76 ^a
Isoleucine	2.21 ± 0.07 ^a	2.49 ± 0.47 ^a
Leucine	3.54 ± 0.24 ^a	4.03 ± 0.74 ^a
Phenylalanine	1.86 ± 0.08 ^a	2.13 ± 0.40 ^a
Hydroxyproline	0.72 ± 0.08 ^a	0.44 ± 0.10 ^a
Cysteine	0.48 ± 0.05 ^b	0.71 ± 0.04 ^a
Methionine	1.50 ± 0.09 ^a	51.07 ± 3.26
Total amino acid	1.62 ± 0.10 ^a	56.28 ± 12.38

^a^b Different letters within the same row denote significant differences ($p<0.05$)

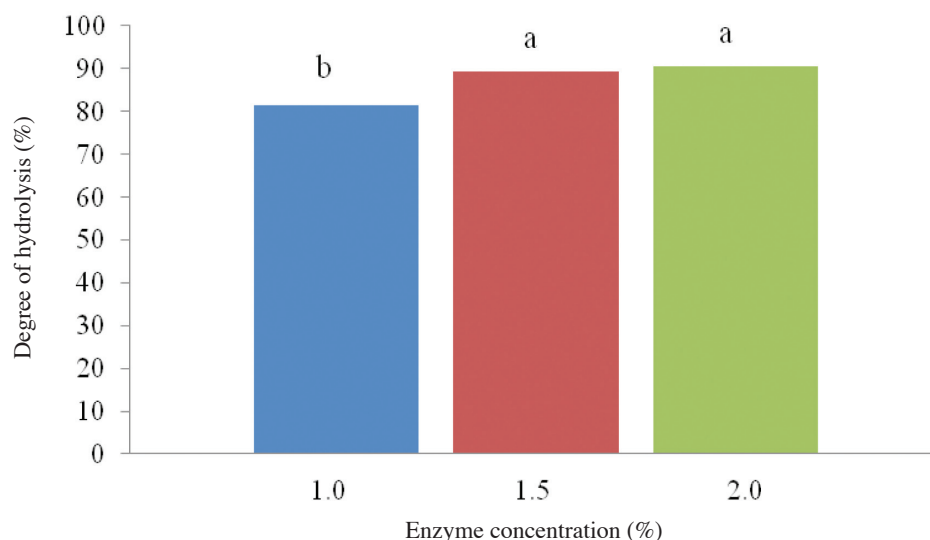


FIGURE 1. The effect of enzyme concentration on hydrolysis of tuna viscera protein extracted at pH 8, 40°C for 60 min incubation time ($n=3$). a-c : means with different letters indicate significant difference ($p<0.05$)

salmon skin reported the same result, where DH increased as the enzyme to substrate level increased.

However, our result gave no significant increment in DH value at 1.5% and 2.0% of alcalase. Liceaga-Gesualdo and Li-Chan (1999) reported that when enzyme is added into a substrate, enzyme will be absorbed into the suspended particles, where the hydrolysis of the enzyme-susceptible peptide linkages will take place simultaneously. After an initial rapid phase of hydrolysis, the rate of hydrolysis will tend to decrease, entering a stationary phase. At this point, increasing the concentration of enzyme used may not result in higher degree of hydrolysis as the concentration of peptide bonds available for hydrolysis may become the limiting factor. However, further studies are needed to determine the validity of this possibility. Bhaskar et al. (2008) also found that DH enters a stationary phase at enzyme levels beyond 1.5% in their optimizing work of protein hydrolysate from viscera waste protein of catla by using alcalase. Yu and Ahmad (1998) also reported the same concentration of enzyme to substrate ratio where the DH showed no significant increment from 16 Au/kg to 20 Au/kg. Subsequently, based on the result obtained for enzyme concentration, alcalase concentration at 1.5% was chosen to be fixed for the next experiment.

The second parameter studied was temperature which involved three different temperatures which were 30°C, 40°C and 60°C. Other parameters used were fixed (1.5% alcalase, 60 min of incubation time and pH8). The percentage of DH were significantly increased ($p<0.05$) when temperature were increased from 30°C to 40°C (Figure 2). However, significant decreased ($p<0.05$) in DH were observed when the temperature was further increased to 60°C. Mukhin and Novikov (2000) reported that for most reactions, rate of hydrolysis increased when temperature increases. In addition, Nielsen (1995) also

reported that exposure of peptide bonds during enzymatic hydrolysis which lead to the increase of DH were caused by the heat treatment. However, at higher temperature the activity of an enzyme can be reduced by its thermal denaturation resulting in a decrease in the hydrolysis rate. The trend obtained for this study on temperature agrees well with those explanations. In addition, Guerard et al. (2002) suggested that at high degrees of hydrolysis, there were formation of reaction products occurred which limit the enzyme activity which also results in a reduction of the reaction rate. However, a decrease in hydrolysis rate may also be due to other factors such as decrease in the concentration of peptide bonds available for hydrolysis, enzyme inhibition and enzyme deactivation. Murano (2003) mentioned that production of hydrolysate is more efficient at temperatures in the range of 32°C-49°C as shown in practice and enzymes are inactivated at high temperature which caused by the unfolding of their structure.

The rate of hydrolysis was slow at 30°C which resulted in a lower DH. This lower rate of hydrolysis may be due to insufficient energy which in this case provided by the temperature for the alcalase to react with the substrates. The similar results were observed by Kristinsson and Rasco (2000) using Atlantic salmon, Guerard et al. (2001) using yellowfin tuna, Ovissipour (2009) using persian sturgeon and Yu and Ahmad (1998) using *Liza subviridis*, a marine fish. From this study, the highest DH observed was at 40°C (95.39%) and was significantly different ($p<0.05$) from DH at 30°C and 60°C. For that reason, temperature at 40°C was chosen to be used in the next stage to determine the effect of pH.

An experiment was then carried out to determine the effect of pH on the DH of tuna viscera hydrolysate. Microbial enzymes like alcalase operating at alkaline pH have been reported to be most efficient in the hydrolysis

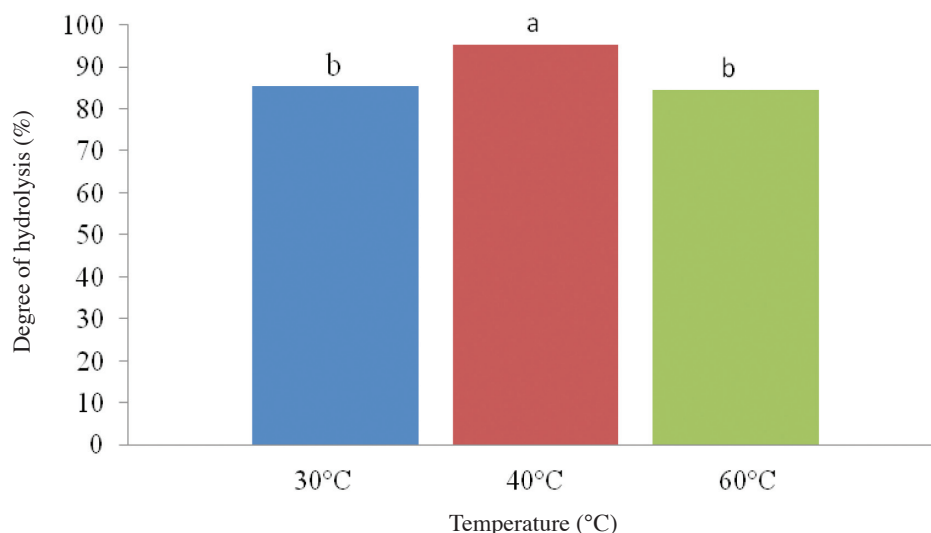


FIGURE 2. The effect of temperature on hydrolysis of tuna viscera protein extracted using 1.5% enzyme concentration at pH8 for 60 min incubation time ($n=3$) a-c : means with different letters indicate significant difference ($p<0.05$)

of fish proteins (Dufosse et al. 1997). For that reason, the value of pH used were 7, 8 and 9, whereas other parameters for enzyme concentration, temperature and incubation time were fixed at 1.5%, 40°C and 60 min, respectively. Our results showed that there was no significant difference ($p>0.05$) observed in DH between pH7, 8 and 9 studied (Figure 3). This result could be explained as the pH value was only adjusted to the desired pH by dissolving the substrate in pre-prepared buffer at the initial stage and was not controlled throughout the hydrolysis process. Changes of pH throughout the process might significantly disturb the efficiency of the enzyme capability in hydrolyzing the protein which most probably the main cause of the result obtained for range of pH.

Yu and Ahmad (1998) reported that in many enzyme-catalyzed reactions, normally pH could cause changes in the reactants among them being the possible denaturation of the protein structure of the enzyme or the disturbance of the ionic character of the substrates which in turns, could affect the ability of the substrate to bind to the enzyme. Many authors had reported successful hydrolysis of plant or animal proteins with alcalase at pH8.0 (Guerard et al. 2001). However, the optimal pH may vary according to the system used in such reaction (i.e: substrate, enzyme concentration). For instance, the optimum pH found for hydrolysing *Liza subviridis* was pH9.0 meanwhile pH8.5 for catla viscera hydrolysis and pH9.5 for hydrolysis of Pacific whiting solid waste (Benjakul & Morrisey 1997; Bhaskar et al. 2008; Yu & Ahmad 1998). In this study, DH between 80 and 85% was obtained when the starting pH values of the tuna viscera protein were 7, 8 and 9. There were no significant changes on percentage of DH among the pH observed. pH8 were chosen to be used in determining effects of time of incubation.

DH was observed to increase significantly ($p<0.05$) when incubation time were increased up to 240 min (Figure

4). Haslaniza et al. (2010) reported that a longer incubation time would allow enzyme to act more extensively on the protein thus increased proteolysis which results in higher DH obtained. In addition, Montecalvo et al. (1984) reported that the increasing DH was also caused by the increased cleavage of peptide bonds which then increases the peptides solubility in TCA. Similar results were reported by several authors such as Bhaskar et al. (2008), Mukhin and Novikov (2001) Ovissipour et al. (2009), where the DH increased with the increase in incubation time.

CONCLUSION

Protein extract (PE) from viscera of tuna can be used as the material of protein hydrolysate due to its high content of protein (78.17 ± 2.91) and low fat (0.23 ± 0.15). The major amino acid composition of the PE were glycine, arginine, alanine, lysine and leucine i.e. 9.6%, 9.2%, 7.0%, 7.2% and 7.0% of the total amino acids, respectively. Based on the results, factors which significantly influenced the percentage of the degree of hydrolysis of protein hydrolysate from tuna viscera PE were enzyme concentration, temperature and time of incubation. With the increase of enzyme concentration from 1.0 to 2.0%, the DH increased significantly. Similarly, increasing the temperature from 30°C to 60°C and time of incubation from 60 min to 240 min also increased the value of DH of protein hydrolysate produced. On the other hand, pH did not affect the DH of tuna viscera protein. As a conclusion, based on the single factor experiment, the highest percentage of DH obtained for tuna viscera hydrolysate was at 1.5% of enzyme concentration, temperature 40°C, pH8 and incubation time of 240 min. Protein hydrolysate from tuna viscera produced with high DH may potentially serve as a good source of desirable peptide and amino acids.

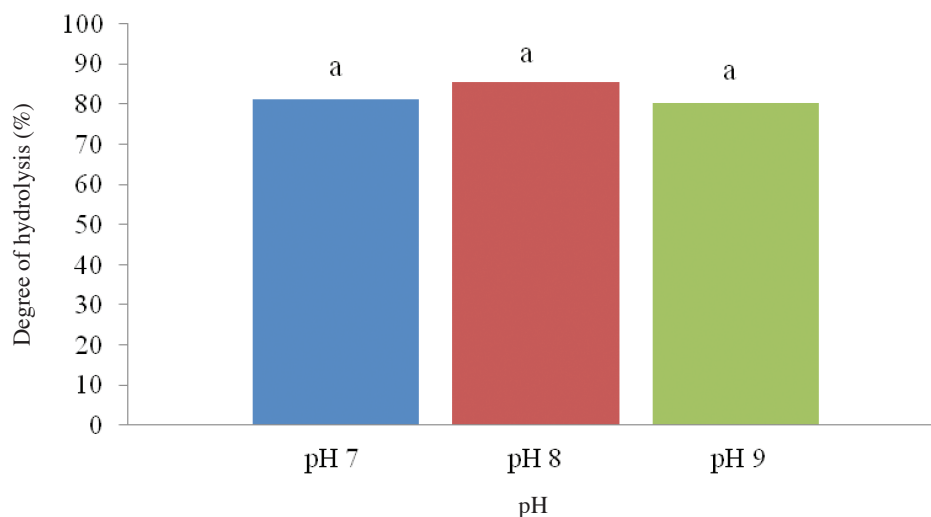


FIGURE 3. The effect of pH on hydrolysis of tuna viscera protein extracted using enzyme concentration of 1.5% at 40°C for 60 min of incubation time ($n=3$). a-b : means with different letters indicate significant difference ($p<0.05$)

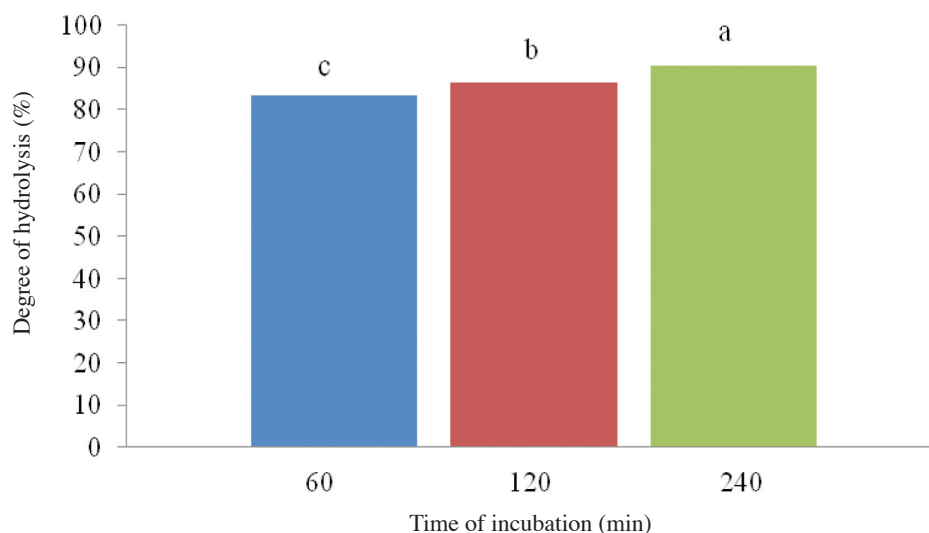


FIGURE 4. The effect of incubation time on hydrolysis of tuna viscera protein extracted using 1.5% enzyme concentration at 40°C and pH8 ($n=3$) a-b : means with different letters indicate significant difference ($p<0.05$)

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