FISH OIL EXTRACTION FROM YELLOWFIN TUNA HEADS BY ENZYMATIC HYDROLYSIS METHOD

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ABSTRACT

A study on the fish oil extraction from yellowfin tuna heads by hydrolysis method using Protamex enzyme was carried out. The parameters of hydrolysis process, fish oil yield and chemical quality of tuna head oil were determined. The study results showed that a considerable amount of oil can be extracted from yellowfin tuna heads. The suitable parameters of enzymatic hydrolysis process for recovering fish oil from yellowfin tuna heads were the water/material ratio of 0.5/1, Protamex concentration of 0.5%, hydrolysis temperature of 55°C and hydrolysis time of 1h. High quality of the yellowfin tuna head oil was obtained from enzymatic hydrolysis. This study suggested that the yellowfin tuna heads generated from tuna processing industry could be utilized as a good source for oil recovery. Tuna head oil could be used as a valuable ingredient both in food and aquaculture feed.

Key words: Enzymatic hydrolysis, fish oil extraction, oil recovery, yellowfin tuna head.

I. INTRODUCTION

Tuna is a valuable source of food and plays an important role in the economy of many countries. Tuna generally is processed as raw fish flesh and marketed as loins. Viet Nam tuna products are exported to the U.S., EU, Japan, ASEAN and other markets (VASEP, 2016). A large amount of by-products consisting of head, bone, viscera, skin and dark muscle is generated from the tuna processing industry (Herpandi et al., 2011). Tuna by-products are perishable due to their high protein and fat contents. Increasing environmental pollution has emphasized the need for better utilization of tuna by-products. Therefore, using the tuna head to recover fish oil is very important to reduce environmental problems. The tuna head oil is an excellent source of omega-3 fatty acids, which are mainly composed of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Nguyen Thi My Huong, 2013). These fatty acids play an essential role in human health and nutrition.

Lipid extraction from many sources and by different methods have been extensively studied (Salam *et al.,* 2005; Gbogouri *et al*., 2006; Batista *et al*., 2009; Khoddami *et al.*, 2012; Ramakrishnan *et al*., 2013). Fish oil is usually extracted from whole fish or fish by-products by chemical process (Mahmoud *et al*., 2008; Norziah *et al.*, 2009), by cooking and pressing (Chantachum *et al*., 2000), or by enzymatic process (Batista *et al*., 2009; Khoddami *et al*., 2012; Ramakrishnan *et al*., 2013). Among the mentioned methods, the enzymatic hydrolysis method used for oil extraction has many advantages, such as the mild hydrolysis conditions, low energy requirement, no use of solvent. The low hydrolysis temperatures minimize the oxidation of polyunsaturated fatty acids. Enzymatic tissue disruption may be a valid alternative technique for releasing natural lipids from fish. During the enzymatic hydrolysis, the combination between lipid and protein was broken down, which lead to fish oil release much easier from fish by-product (Qi-yuan *et al*., 2016).

The purpose of this study was to determine the suitable hydrolysis conditions for oil recovery from yellowfin tuna heads using Protamex and to value the chemical quality of tuna head oil with various parameters, including free fatty acid, acid value, peroxide value, iodine value and saponification value.

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II. MATERIALS AND METHODS

1. Materials

Yellowfin tuna (Thunnus albacares) heads were provided by Thinh Hung, a seafood processing company in Nha Trang, Vietnam. Yellowfin tuna heads were stored with crushed ice at 0 - 4°C in a polystyrene box and transported immediately to the laboratory of Nha Trang university. After their arrival, they were washed and ground. The minced tuna heads were packed in plastic bags, frozen and stored at -20°C until their use (approximately a month).

2. Enzyme

The enzyme used for the hydrolysis of yellowfin tuna heads was Protamex, which was produced by Novozymes (Denmark). Protamex is a Bacillus protease complex. The declared activity of Protamex is 1.5 AU/g. Optimal working conditions of Protamex are at pH 5.5-7.5 and 35-60°C.

3. Determination of suitable hydrolysis conditions for oil extraction from yellowfin tuna head

3.1. Determination of suitable water/material ratio

In order to determine the suitable water/material ratio for oil recovery, the minced tuna heads were hydrolyzed by using 0.5% Protamex in 2h at temperature of 50°C, pH 6.5 with water/material ratios of 0/1, 0.25/1, 0.5/1, 0.75/1 and 1/1. After hydrolysis, the enzyme was inactivated by heat treatment at 90°C for 10 minutes in a water bath. Then, the mixture was filtered through a mesh to remove the solid fraction (bones). The filtrate was centrifuged at 10000 rpm at 4°C for 30 minutes. After centrifugation, the following four fractions were formed: the oil fraction on the top, the emulsion and the liquid protein hydrolysate in the middle and the sludge on the bottom. The oil fraction was recovered, then weighed to calculate the percentage of recovered oil. The acid value and peroxide value of oil were determined. From obtained results, the suitable water/material ratio was selected.

3.2. Determination of suitable enzyme concentration

With the suitable water/material ratio identified and hydrolysis conditions as above, the minced tuna heads were hydrolyzed with 0.1%, 0.3%, 0.5%, 0.7% and 0.9% Protamex. After hydrolysis, the same steps as above were carried out. The suitable enzyme concentration was selected.

3.3. Determination of suitable hydrolysis temperature

With the suitable water/material ratio and enzyme concentration identified, the minced tuna heads were hydrolyzed in 2h at pH 6.5 and temperature of 45°C, 50°C, 55°C and 60°C. After hydrolysis, the same steps as above were carried out. The suitable hydrolysis temperature was selected.

3.4. Determination of suitable hydrolysis time

With the suitable water/material ratio and enzyme concentration identified, the minced tuna heads were hydrolyzed at pH 6.5 and suitable hydrolysis temperature identified in 0.5h; 1h; 2h; 3h and 4h. After hydrolysis, the same steps as above were carried out. The suitable hydrolysis time was selected.

4. Chemical analyses

Lipid content was determined according to the method of Folch *et al*. (1957). The free fatty acid content, acid value, peroxide value, iodine value, saponification value were determined according to American Oil Chemists' Society AOCS (1997).

5. Oil recovery

The oil obtained was weighed using a digital balance (Precisa-Model XT 2200c). The percentage of recovered oil from yellowfin tuna head was calculated as follows:

$$Oil\ recovery\ (\%) = \frac{Weight\ of\ recovered\ \ oil}{Weight\ of\ sample\ x\ \ lipid\ content\ of\ tuna\ head}\ x\ 100$$

6. Statistical analysis

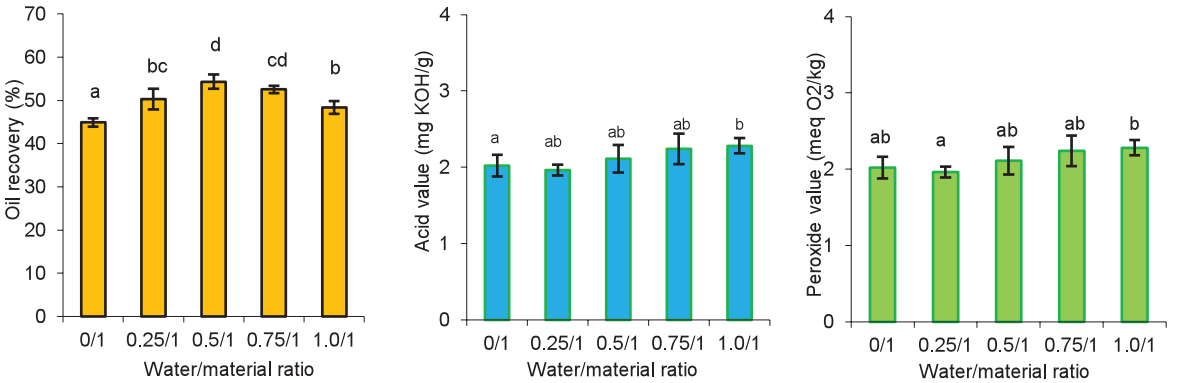
The experiments were carried out in triplicates. The obtained data were subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significant difference between samples at P<0.05 level using the SPSS 15.0 programme.

III. RESULTS AND DISCUSSION

1. Determination of suitable hydrolysis conditions for oil extraction from yellowfin tuna head

1.1. Determination of suitable water/material ratio

The influence of water/material ratio on the oil recovery, acid value and peroxide value of tuna head oil is shown in Figure 1.



(a) (b) (c)

Figure 1. The influence of water/material ratio on the oil recovery (a), acid value (b) and peroxide value (c) of yellowfin tuna head oil.

Enzymatic hydrolysis resulted in formation of four phases: an oily phase, emulsion phase, aqueous phase and sludge. The results indicated that the water/material ratio had a significant effect on the oil recovery (Figure 1a). The oil recovery reached the highest value (54.4%) with water/material ratio of 0.5/1. Qi-yuan *et al* (2016) showed that the maximum oil recovery from mackerel viscera was 78.66%. The oil recovered from salmon heads using Bromelain and Protex were 65% and 88%, respectively (Mbatia *et al*, 2010).

The oil recovery reduced with the increase in water/material ratio from 0.5/1 to 1/1. Mbatia *et al*. (2010) also reported that an increase in water/material ratio during the hydrolysis resulted in a decrease in oil yield. Decrease in oil yield with increasing water/material ratio during the hydrolysis could have been due to emulsion formation (Mbatia *et al*., 2010).

The acid value (Figure 1b) and peroxide value (Figure 1c) of the tuna head oil tended to increase with the raise of water/material ratio. However, the increases in acid value and peroxide value of oil were not significant. The oil extracted from yellowfin tuna heads had the highest acid value (3.20 mg KOH/g) and the highest peroxide value (2.28 meq O_2/kg) when water/material ratio was 1/1. The acid value indicates the formation of free fatty acids because of oil hydrolysis. Ahmed et al (2017) showed that the acid values of the oil extracted from bigeye tuna by-products ranged from 4 to 7.4 mg KOH/g. The peroxide value of the oil extracted from sardine tissue was 2.78 meq O_2/kg (Pravinkumar *et al*., 2015).

The study indicated that the enzymatic hydrolysis using Protamex with water/material ratio of 0.5/1 has brought the highest percentage of oil recovery. Therefore, the water/material ratio of 0.5/1 was suitable for oil recovery from yellowfin tuna head.

1.2. Determination of suitable enzyme concentration

During the enzymatic extraction of oil from the yellowfin tuna heads with Protamex, enzyme concentration plays an important role in the recovery of oil. Figure 2 indicate the influence of enzyme/material ratio on the release of oil, acid value and peroxide value of tuna head oil.

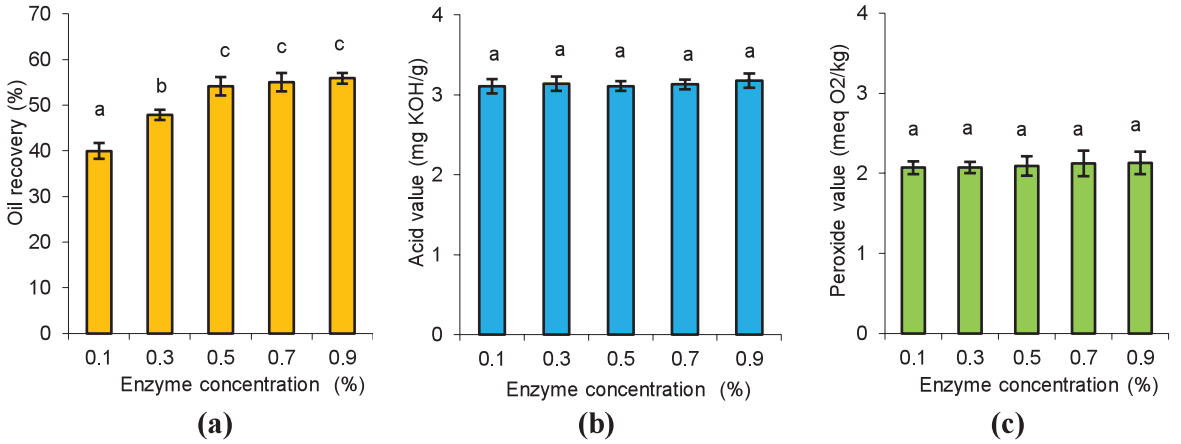


Figure 2. The influence of enzyme concentration on the oil recovery (a), acid value (b) and peroxide value (c) of yellowfin tuna head oil

The results demonstrated that increasing enzyme concentration increased the oil recovery from tuna heads (Figure 2a). The oil recovery increased strongly ($P<0.05$) from 39.9% to 54.1% with the increase of the enzyme concentration from 0.1 to 0.5%. However, there were no significant differences in oil recovery among the samples with the enzyme concentrations of 0.5%, 0.7% and 0.9%. Ramakrishnan *et al* (2013) also indicated that increasing the enzyme concentration increased the oil recovery from mackerel head. For the 0.5% enzyme concentration and 1 hour hydrolysis time, the oil recovery from mackerel head was 55.82%. When the enzyme concentration was increased from 0.5% to 1%, the oil recovery increased from 55.82 to 56.96%. Mbatia *et al*. (2010) stated that maximum oil recovery from salmon heads was achieved when 0.5% Bromelain was used. A higher enzyme concentration did not result in further increase in oil recovery.

There were not significant differences in acid values (Figure 2b) and peroxide values (Figure 2c) of the fish oil obtained among the samples with the different enzyme concentrations. This mean that the enzyme concentration did not significantly affect on the acid value and peroxide value of the oil extraced from yellowfin tuna heads. The acid values of the oil extracted from hilsa fish *(Hilsa ilisha)* by-products ranged from 4.16 to 12 mg KOH/g (Salam *et al*., 2005). According to Khoddami *et al* (2012), the peroxide value of the oil extracted from tuna *(Euthynnus affinis)* head was 7.31 meq O_2/kg.

According to the results in this study, the highest oil recovery was obtained with enzyme concentration of 0.5%. A higher enzyme concentration did not improve the oil recovery as well as acid value and peroxide value. Therefore, the enzyme concentration of 0.5% was suitable for the oil extraction in order to reduce the cost associated with the enzyme.

1.3. Determination of suitable hydrolysis temperature

The influence of hydrolysis temperature on the oil recovery, acid value and peroxide value of tuna head oil is demonstrated in Figure 3.

The results indicated that the hydrolysis temperature had a significant effect ($P<0.05$) on the oil recovery (Figure 3a). Increasing the hydrolysis temperature led to increase the oil recovery from tuna heads. The oil recovery increased sharply from 48.6% to 59% with the hydrolysis temperatures in a range of 45-55°C. The highest oil recovery (59%) was achieved at 55°C. However, with the hydrolysis temperature of 60°C, the oil recovery from tuna head decreased to 50.9%. This may be due to decreasing the activity of enzyme Protamex at 60°C. Deepika et al (2014) showed that the highest oil recoveries from the salmon gut, heads and frame were 80.01%, 59.92% and

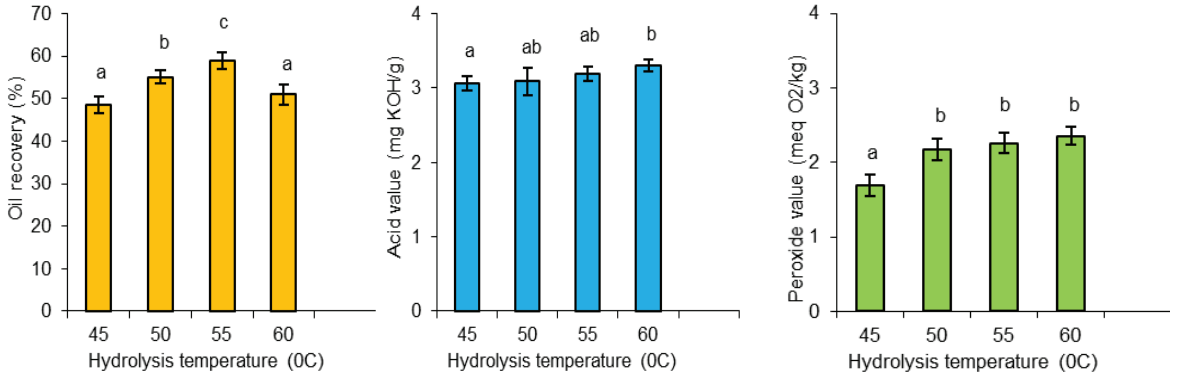


Figure 3. The influence of hydrolysis temperature on the oil recovery (a), acid value (b) and peroxide value (c) of yellowfin tuna head oil

78.58%, respectively.

The acid value (Figure 3b) and peroxide value (Figure 3c) of the tuna head oil slightly increased from 3.06 to 3.31 mg KOH/g and from 1.69 to 2.35 meq O_2/kg, respectively when the temperature increased from 45°C to 60°C. The higher extraction temperatures led to fish oil with higher acid value. Increasing extraction temperature can cause faster lipid degradation to form free fatty acids. Deepika et al. (2014) reported that the oil extracted from salmon heads and frame at 30°C and 40°C had low acid values (0.33-2.10 mg KOH/g). However, the acid values of the oil extracted at 30°C and 40°C from salmon gut were 12.91 and 17.49 mg KOH/g, respectively. Deepika et al. (2014) also showed that the peroxide value of all oil samples extracted at different temperatures and reaction time were between 0.28-2.65 meq/kg.

The results showed that the suitable temperature for oil extraction from yellowfin tuna heads was 55°C.

1.4. Determination of suitable hydrolysis time

During the enzymatic extraction of oil with protease, the hydrolysis time plays an important role in the oil recovery from the tuna head and quality of oil (acid value and peroxide value). The influence of hydrolysis time on the oil recovery, acid value and peroxide value of tuna head oil is shown in Figure 4.

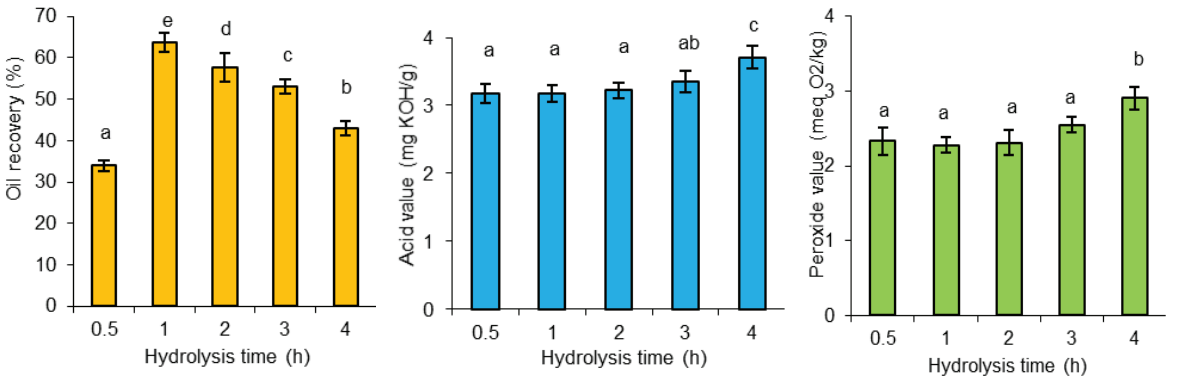


Figure 4. The influence of hydrolysis time on the oil recovery (a), acid value (b) and peroxide value (c) of yellowfin tuna head oil

The results indicated that there was a significant increase in oil recovery during the first hour, followed by a decrease during the next 3 hours (Figure 4a). The oil recovery from tuna heads was 34% after 0.5h of hydrolysis and reached the highest value (63.7%) after 1h of hydrolysis. However, when the hydrolysis

time prolonged over 1h, the free oil recovery decreased significantly. After 4h of hydrolysis, the oil recovery only attained 43%.

These results implied that the hydrolysis time of 0.5h was not sufficient to release the oil. That led to a low percentage of oil recovery. The hydrolysis time of 1h was sufficient to release a large amount of free oil from tuna heads. The decrease in amount of free oil after 1h may be due to interaction of released oil with hydrolyzed proteins during hydrolysis as showed by Šližyte *et al*. (2005). Mbatia *et al*. (2010) also reported the initial stage of hydrolysis could be sufficient to release the lipids. The longer hydrolysis time did not improve the oil recovery. According to Dumay *et al*. (2009), it is not beneficial to perform a long hydrolysis to obtain the highest oil release. Indeed, the tissue disruption obtained at the beginning of the proteolysis appears sufficient to release the lipids.

The acid values (Figure 4b) and peroxide values (Figure 4c) of tuna head oil were not significantly different among the samples with the hydrolysis time from 0.5 to 3h. The highest acid value and peroxide value were obtained after 4h of hydrolysis. This may be due to the hydrolysis and oxidation of released oil with the long time of hydrolysis.

The study results suggested that the suitable hydrolysis time for oil extraction was 1h.

In brief, the suitable hydrolysis conditions for oil extraction from yellowfin tuna heads in this study were the water/material ratio of 0.5/1, enzyme concentration of 0.5%, hydrolysis temperature of 55°C and hydrolysis time of 1h.

2. Chemical quality of the oil extracted from yellowfin tuna heads

Yellowfin tuna heads were hydrolyzed with the suitable hydrolysis conditions determined, the oil recovery from yellowfin tuna heads was 63.7 ± 0.8%. In order to assess the quality of oil extracted from yellowfin tuna heads, some chemical properties including free fatty acid content, acid value, peroxide value, iodine value and saponification value were determined. Chemical quality of yellowfin tuna head oil is shown in Table 1.

Table 1. Chemical quality of oil extracted from yellowfin tuna heads

Chemical quality	Content
Free fatty acid (%)	1.56 ± 0.22
Acid value (mg KOH/g)	3.12 ± 0.34
Peroxide value (meq O_2/kg)	2.24 ± 0.25
Iodine value (g I_2/100g)	177 ± 3
Saponification value (mg KOH/g)	185 ± 3

The free fatty acid content in oil is one of the most important quality parameters to evaluate the quality of oil because the free fatty acid are more susceptible to oxidation than esterified fatty acids (Ahmed *et al*.,2017). The lower free fatty acid content ensures higher grade quality with fewer changes for further oxidation. As quality specifications for crude fish oil, Bimbo (1998) reported that the free fatty acid content should range between 1 and 7% but usually ranges between 2 and 5%. The result in this study (Table 1) demonstrated that the amount of free fatty acid in the yellowfin tuna head oil was low (1.56%). This value was much lower than that of the oil extracted from head of tuna *Euthynnus affinis* (4.08%) studied by Khoddami *et al*. (2012).

The acid value is a measure of the lipid hydrolysis that had occurred in the oil and is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 1g of oil. The acid value of yellowfin tuna head oil was found to be 3.12 mg KOH/g, which is below the acceptable limit of 7-8 mg KOH/g reported by Bimbo and Crowther (1991).

The peroxide value is commonly used to determine the rancidity of oil and is expressed in milli equivalent of active oxygen per kg of oil. The maximum limit of peroxide value of

crude oil is 8 meq O_2/kg to be acceptable for human consumption (Boran *et al*., 2006). The oil extracted from yellowfin tuna head had a peroxide value of 2.24 meq O_2/kg, which was still within the acceptable quality limit. This indicated that the extracted fish oil had low lipid oxidation rate. According to Khoddami *et al*. (2012), the peroxide value of oil from head of tuna *Euthynnus affinis* was 7.31 meq O_2/ kg. Bimbo (1998) reported that the peroxide value of crude fish oil was between 3 to 20 meq O_2/kg.

The iodine value is a measure of degree of unsaturation of the oil and is defined as grams of iodine absorbed by 100 g of oil. Yellowfin tuna head oil had a iodine value of 177 g I_2/100g, which was higher than that of mackerel oil (134 g I_2/100g) (Zuta *et al*., 2003). This indicated that the oil from yellowfin tuna head contains a high amount of unsaturated fatty acids.

Saponification is the process of breaking down a neutral oil into glycerol and fatty acids by alkali treatment. Saponification value represents the number of milligrams of potassium hydroxide required to saponify 1 g of oil. The oil extracted from yellowfin tuna heads had a saponification value of 185 mg KOH/g, which was similar to that of sardine oil (186.85 mg KOH/g) reported by Noriega-Rodríguez *et al*. (2009). Saponification values of the hilsa fish oils from different parts were found to be arranged from 180.28 to 194 (Salam *et al*., 2005).

IV. CONCLUSION

The effects of the hydrolysis conditions on the extraction of oil from the yellowfin tuna heads were studied. The suitable parameters for oil extraction from yellowfin tuna heads were the water/material ratio of 0.5/1, enzyme concentration of 0.5%, hydrolysis temperature of 55°C and hydrolysis time of 1h. With these suitable hydrolysis conditions, the oil recovery from yellowfin tuna heads was 63.7%. The oil obtained after enzymatic hydrolysis had a good quality with acid value of 3.12 mg KOH/g and peroxide value of 2.24 (meq O_2/kg). Tuna head oil could be used as a valuable ingredient both in food and aquaculture feed.

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