

## **EFFECT OF LIME AND TAMARIND PRE-TREATMENT ON THE ENZYMATICALLY PRODUCED PROTEIN HYDROLYSATE FROM SILVER CATFISH (*Pangasius sutchi*) FLESH**

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### **Abstract**

One of the main obstacles of protein hydrolysate application in food is bitterness and fishy off-flavor. Pre-treatment of the raw materials prior to hydrolysate production is one way of preventing the development of this undesirable flavor. In this study, silver catfish which was used as the raw material was soaked in lime or tamarind juice prior to hydrolysis process to produce hydrolysate with reduce bitterness and fishy off-flavor. Initially, the fish flesh was pre-treated by soaking in either water, lime juice (*Citrus aurantifolia*) or tamarind juice (*Tamarindus indica*). After the pre-treatment, the homogenised flesh was hydrolysed in Flavourzyme 500L at pH 7, 50°C and enzyme substrate ratio (ES) of 2% for 120 minutes to produce control (CH), lime (LIH) and tamarind (TAH) hydrolysates after soaking in water, lime juice and tamarind juice, respectively. The physicochemical and sensory properties of the hydrolysates were compared. Soaking in tamarind juice resulted in significantly ( $p < 0.05$ ) higher degree of hydrolysis (84.2% DH) than soaking in lime juice with no significant difference ( $p > 0.05$ ) in yield. TAH was significantly ( $p < 0.05$ ) darker than others and characterised by 96.16% nitrogen solubility index (NSI), 5.44% moisture and 68.28% protein content. Sensory analysis by Quantitative Descriptive Analysis (QDA) showed that TAH had fishy flavor, fishy odor, umami and bitterness intensity ranking between 'weak' and 'moderate' and sweetness between 'moderate' and 'strong'. Overall, soaking in tamarind juice is an effective pre-treatment method to produce silver catfish hydrolysate with low intensity of fishy flavor and odor.

**Keywords:** silver catfish, lime, tamarind, hydrolysate, flavourzyme

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### **Introduction**

Silver catfish is known as *Pangasius sutchi*, it is a popular freshwater fish in Malaysia. Silver catfish accounts for 36.7% of total freshwater aquaculture production and the estimated retail value of silver catfish production from freshwater culture system in 2010 was about RM 309,122.08 (Abbas *et al.*, 2006; Department of Fisheries Malaysia, 2010). This species is widely spread in rivers of Burma, India, Cambodia, Thailand and Malaysia (Abbas *et al.*, 2006).

Fish converted by hydrolysis into a more marketable and functional form is called fish protein hydrolysate. Fish protein hydrolysates have variety of industrial applications such as protein supplements, beverage stabilizers, milk replacers and flavor enhancers (Ghaly *et al.*, 2013). Hydrolysis of protein can be achieved by using acid, alkali or enzyme which breaks the parent proteins into peptides, free amino acids and smaller protein fractions (Brar *et al.*, 2014). Among these, enzymatic hydrolysis is more preferred due to lesser development of undesirable products, mild reaction conditions, high yield and high product quality (Roslan *et al.*, 2014).

Hydrolysis of protein has been shown to induce flavor defects such as off-flavor and bitterness (Nilsang *et al.*, 2005; Spellman *et al.*, 2009; Hou *et al.*, 2011). Previous study showed that when green mussels were hydrolysed with alcalase at either pH 7, E/S 5% or pH 9, E/S 3% at 60°C for two hours, both hydrolysates had slightly bitter taste (Normah *et al.*, 2013). It was also reported that threadfin bream (*Nemipterus japonicus*) hydrolysed with alcalase at pH 8.5, 60°C, 2% for 2 hours had a combination of bitter, umami, salty, sweet and sour tastes with fishy flavor (Normah *et al.*, 2005). Also, fish soluble concentrate (FSC), a by-product from canned fish industry hydrolyzed using Flavourzyme exhibited a fish-like and dry squid-like odor and diluted fish sauce-like taste (Nilsang *et al.*, 2005). Ethanol extraction of herring protein with a fish/ ethanol (90%) ratio of 1:2 (w/v) at 70°C for 30 min reduced the bitterness and unpleasant fishy odor of hydrolysate to barely detectable levels (Hoyle and Merritt 1994).

The major drawback of fish protein hydrolysate application in food is the fishy odor which makes it unfit for human consumption unless the smell is removed (Kim, 2014). It is believed that traditional methods such as washing fish with flour, vinegar, milk, salt, lime juice and tamarind juice help remove the fishy smell and increase the acceptability of the fish (Jamilah and Siti Aini, 1997; Korten, 2015). Currently, there is no published information regarding the use of lime and tamarind as pre-treatment step in reducing the fishy flavor and fishy odor of fish protein hydrolysate. In order to expand fish hydrolysate application in food, further treatment to produce hydrolysate with insignificant fishy odor should be done. This study was carried out to determine the effect of pre-treatment of silver catfish flesh in lime or tamarind solution on the sensory and physicochemical characteristics of silver catfish hydrolysate.

## Materials and Methods

### Materials

Silver catfish which was purchased from Temerloh, Pahang, was transported to the laboratory in box filled with ice and immediately stored at -21°C upon arrival. Flavourzyme with declared activity of 500 LAPU/g was purchased from Chemolab Supplies, Malaysia. All chemicals used were of analytical grade and obtained from Sigma (Sigma Chemical Co., St Louis, MO, US). Lime (*Citrus aurantifolia*) at the commercial green maturity stage was purchased from nearby hypermarket. Monosodium glutamate (Ajinomoto (M) Bhd.) and commercial fish sauce were obtained from the local hypermarket while food-grade caffeine from LabChem Sdn. Bhd., Malaysia.

### Preparation of soaking solutions

Lime juice was prepared by squeezing lime (*Citrus aurantifolia*) and diluting it at the ratio of 1:1 v/v with distilled water. Tamarind juice was prepared by diluting tamarind (*Tamarindus indica*) with distilled water at a ratio of 1:1 w/v.

### Preparation of silver catfish (*Pangasius sutchi*) protein hydrolysates

Hydrolysates were prepared according to Normah *et al.*, (2005). However, the fish flesh was pre-treated by soaking in water (control), lime juice at 1:2 w/v (flesh to juice) or tamarind at 1:2 w/v (flesh to juice) for 15 minutes at room temperature prior to hydrolysates preparation. Hydrolysates were prepared by hydrolysing the minced pre-treated fish flesh in Flavourzyme 500L at pH 7, 50°C and enzyme-substrate ratio (ES) of 2% for 120 minutes. The hydrolysates were freeze-dried after hydrolysis to obtain the powdered form. Hydrolysates obtained after the pre-treatment and hydrolysis were denoted as 'CH' for

pre-treatment with water, 'LIH' for pre-treatment with lime juice and 'TAH' for pre-treatment with tamarind juice.

#### Determination of degree of hydrolysis (DH)

DH was determined by using a pH stat method based on the amount of NaOH consumed (Adler-Nissen, 1986). Calculation of degree of hydrolysis was summarized in the equation below:

$$\% DH = \frac{\beta \times N\beta}{\alpha \times M_p \times htot} \times 100$$

where,

$\beta$  = amount of NaOH consumed (mL) during the hydrolysis

$N\beta$  = normality of NaOH

$M_p$  = mass (g) of protein ( $N \times 6.25$ )

$\alpha$  = average degree of dissociation of the  $\alpha$ -NH<sub>2</sub> groups

$htot^*$  = total number of peptide bonds in the protein substrate

\* $htot$  value was obtained as described in Adler-Nissen, 1986.

#### Yield

Yield was calculated based on the ratio of hydrolysate mass and the total weight of the flesh as follows:

$$Yield (\%) = \frac{\text{weight of powdered hydrolysate}}{\text{weight of flesh}} \times 100$$

#### Color measurement

Chromameter instrument (CR 400, Kinoca Minolta, Japan) was used to measure the hydrolysate's color which is in powdered form. L\*, a\* and b\* parameters indicate lightness, redness-greenness and blueness-yellowness, respectively. Each run was repeated three times.

#### Chemical analysis of the hydrolysate

Moisture and protein content were determined according to AOAC (2000). Moisture was measured using the oven dried method while protein content using the Kjeldahl method.

#### Nitrogen Solubility Index (NSI)

NSI of the hydrolysate was determined according to Morr *et al.* (1985). Nitrogen content was measured according to AOAC (2000). The nitrogen solubility index (NSI) was calculated as:

$$NSI (\%) = \frac{\text{protein content in supernatant}}{\text{protein content in sample}} \times 100$$

#### Emulsifying properties

Emulsifying capacity was determined according to the method by Vioque *et al.*, (2000) with slight modifications. 1 g of hydrolysate was homogenised with 50 ml of water at 1000 rpm for 30 seconds (HG-15D, Homogenizer Wise Mix, Malaysia). 25 ml corn oil was then added and the mixture was homogenised for another 30 seconds. Another 25 ml of corn oil was added and the mixture was homogenised again for 90 seconds. The emulsion was centrifuged at 5000 rpm for 4 minutes (Eppendorf

5418 microcentrifuge, United States). The final volume of oil after centrifugation was measured. Emulsifying activity was calculated as follows:

$$\text{Emulsifying Capacity (\%)} = \frac{(\text{Initial oil volume} - \text{oil volume after centrifuge}) \times 100}{\text{Initial oil volume}}$$

Emulsifying stability was determined by heating the mixture at 85 °C for 15 minutes, cooled to room temperature and centrifuged at 1100 rpm for 3 minutes. Volume of oil after heating was determined. Emulsion stability was calculated as follows:

$$\text{Emulsifying Stability (\%)} = \frac{\text{Volume of oil layer after heating}}{\text{Volume of oil layer before heating}} \times 100$$

### **Water and oil holding capacity**

Water and oil holding capacity of hydrolysate were determined according to the method by Vioque *et al.*, (2000) with some modifications. 0.5 g hydrolysate was stirred with 10 ml distilled water or corn oil and centrifuged at 5727 rpm for 30 min (Eppendorf 5418 microcentrifuge, United States). The final volume of distilled water or corn oil was measured. The water or oil holding capacity was calculated as follows:

$$\text{Water absorption (ml/g)} = \frac{(\text{Initial volume distilled water} - \text{final volume distilled water})}{\text{Mass of hydrolysate}}$$

### **Sensory analysis**

A quantitative descriptive analysis (QDA) was performed according to Nilsang *et al.* (2005) involving ten panelists who were semi-trained for fishy flavor, fishy odor, umami, sweetness and bitterness prior to the sensory evaluation. Different concentrations of fish sauce in distilled water (30, 50, 80 and 100% v/v) were provided during the training to determine the intensity of fishy odor and fishy flavor, monosodium glutamate (MSG) (30, 50, 80 and 90% w/v) for umami, sugar (30, 50, 80 and 90% w/v) for sweetness and caffeine (0, 0.075, 0.15 and 0.30% w/v) for bitterness. The concentration with the lowest intensity that panelists could perceive was identified and this concentration was used as the reference solution during the sensory session to evaluate the intensity for each attribute. A 15-cm line scale anchored from 'absent' to 'very strong' was defined.

During the evaluation session, each panelist was served with 2 ml hydrolysate solution in a capped sensory cup coded with three digit random numbers together with the reference solutions. A 15-cm line scale anchored from 'absent' to 'very strong' was provided. Mineral water was used for rinsing while coffee beans to cleanse their olfactory palate between each sample evaluation.

### **Statistical analysis**

The obtained data were subjected to analysis of variance (ANOVA). The mean comparisons were carried out using Duncan multiple range tests. Statistical analysis was performed using the Statistical Analysis System (SAS, 2004).

## **Results and Discussion**

### **Degree of hydrolysis**

Soaking in tamarind solution resulted in significantly higher ( $p < 0.05$ ) DH (84.20%) followed by lime (81.54%) and control (48.14%) (Table 1). Higher DH in both treated samples may be related to the acidity of the soaking solutions. The pHs for lime and tamarind juice are 3.32 and 3.05, respectively. The initial

pH of the hydrolysis mixture without any pre-treatment was 6.68, while the hydrolysis mixture in which the flesh was pre-treated with lime and tamarind juice had the pH of 4.56 and 4.24, respectively.

Table 1. Physicochemical properties of silver catfish (*Pangasius sutchi*) hydrolysate prepared after pre-treatment by soaking in water (CH), lime juice (LIH) or tamarind juice (TAH)

	Samples			
	Flesh	CH	LIH	TAH
Degree of hydrolysis (%)	-	48.14±0.98 <sup>c</sup>	81.54±0.98 <sup>b</sup>	84.20±0.43 <sup>a</sup>
Yield (%)	-	4.39±0.13 <sup>b</sup>	5.74±0.07 <sup>a</sup>	5.78±0.12 <sup>a</sup>
Color				
L*	-	88.47±0.52 <sup>b</sup>	87.82±0.41 <sup>c</sup>	89.52±0.20 <sup>a</sup>
a*	-	-0.44±0.02 <sup>a</sup>	-1.02±0.01 <sup>c</sup>	-0.87±0.04 <sup>b</sup>
b*	-	13.77±0.13 <sup>a</sup>	7.76±0.20 <sup>c</sup>	11.2±0.18 <sup>b</sup>
Moisture (%)	81.10±2.90 <sup>a</sup>	4.16±0.53 <sup>c</sup>	4.85±0.38 <sup>bc</sup>	5.44±0.23 <sup>b</sup>
Protein (%)	13.45±0.07 <sup>d</sup>	73.99±0.46 <sup>a</sup>	68.28±1.46 <sup>b</sup>	61.78±0.86 <sup>c</sup>
Nitrogen solubility index (%)	-	71.85±1.10 <sup>c</sup>	93.08±1.68 <sup>b</sup>	96.16±0.92 <sup>a</sup>
Emulsifying capacity (%)	-	13.04±1.51 <sup>a</sup>	11.81±1.26 <sup>a</sup>	9.14±1.62 <sup>b</sup>
Emulsifying stability (%)	-	60.0±2.08 <sup>a</sup>	52.83±2.18 <sup>b</sup>	49.98±3.42 <sup>c</sup>
Water holding capacity (ml/g)	-	2.10±0.04 <sup>a</sup>	2.04±0.03 <sup>b</sup>	1.64±0.02 <sup>c</sup>
Oil holding capacity (ml/g)	-	3.06±0.03 <sup>a</sup>	2.79±0.08 <sup>b</sup>	2.21±0.10 <sup>c</sup>

Means within rows followed by different superscript a, b or c are significantly different at  $p < 0.05$ .

Thus, based on the initial pH of the hydrolysis mixture, TAH required higher amount of sodium hydroxide to be added than other hydrolysates to maintain the pH (pH 7) throughout the hydrolysis process. With pH-stat method, when hydrolysis is carried out at neutral or alkaline conditions, dissociation of protons from the free amino groups released is favored (Adler-Nissen, 1986). Protons freed into the surrounding medium lowers the pH of the reaction mixture. Thus, addition of higher amount of sodium hydroxide resulted in higher DH.

### Yield

Yield of pre-treated hydrolysates was significantly higher than control ( $p < 0.05$ ) and increased along with DH (Table 1). Raw material containing high amount of lipids gave low percentage of solubilised protein (Slizyte *et al.*, 2005). The acidity of the soaking solutions may have reduced the amount of lipid present in silver catfish flesh, thus, contribute to higher yield for the treated hydrolysate.

### Color measurement

The lightness (L\*), redness (a\*) and yellowness (b\*) values of the silver catfish hydrolysates are shown in Table 1. LIH had the lowest L\*, a\* and b\* values (L\* = 87.82, a\* = - 1.02, b\* = 7.76) and was less yellowish than control and TAH. The acidity of lime juice may act as a natural bleaching agent that reduces the yellowish color. Meanwhile, the natural color of tamarind juice may have influenced the color of TAH (b\*=11.2). Previous studies demonstrated that round scad protein hydrolysate prepared using prepared using flavourzyme was brownish yellow (L\* = 58.00, a\* = 8.38, b\* = 28.32) (Thiansilakul *et al.*, 2007).

### Chemical analysis of hydrolysate

The moisture and protein content of silver catfish flesh and hydrolysate are shown in Table 1. All the hydrolysates had significantly lower ( $p < 0.05$ ) moisture content than the flesh ranging between 4.16 to 5.44%. The moisture content of silver catfish flesh (81.10%) was in agreement with the range stated previously which is 80-85% (Orban *et al.*, 2008). Most fish protein hydrolysates contain moisture below

10% (Thiansilakul *et al.*, 2007; Ovissipour *et al.*, 2009; Chalamaiah *et al.*, 2010; Foh *et al.*, 2011). Low moisture content (< 6%) contributes to hydrolysate stability (Tanuja *et al.*, 2012).

All the hydrolysates showed significantly higher ( $p < 0.05$ ) protein content than the flesh (Table 1). The flesh contains 13.45% protein which is within 12.6 to 15.6% as reported by Orban *et al.*, (2008). Previous studies demonstrated that most fish protein hydrolysates had protein content ranged between 60 to 90% (Thiansilakul *et al.*, 2007; Choi *et al.*, 2009; Chalamaiah *et al.*, 2010). High protein content demonstrates the hydrolysate potential application as protein supplements for human nutrition (Chalamaiah *et al.*, 2012). The protein content was high as a result of the removal of insoluble undigested non-protein substances, the solubilisation of protein during hydrolysis and the partial removal of lipid after hydrolysis (Thiansilakul *et al.*, 2007). Both the pre-treated hydrolysates had significantly lower ( $p < 0.05$ ) protein content than the control. Similarly, cape hake protein hydrolysate prepared using alcalase had a protein content of 86.9% while the similar hydrolysate that was pre-treated with citric acid and calcium chloride had lower protein content (66.3%) (Pires *et al.*, 2015). Flavourzyme had optimum pH within pH 5.0-7.0. The initial pH of the lime pre-treated hydrolysis mixture (pH 4.56) and tamarind pre-treated hydrolysis mixture (pH 4.24) may have reduced the effectiveness of the flavourzyme in breaking down the fish protein into smaller peptides. Furthermore, soaking of fish flesh in lime or tamarind juice prior to hydrolysis could have contributed to the denaturation of fish protein due to their acidity.

#### **Nitrogen solubility index (NSI)**

Both LIH and TAH had significantly ( $p < 0.05$ ) higher NSI than the control. Hydrolysis process increases the solubility of protein hydrolysates due to the reduction of its secondary structure and release of smaller polypeptide units from the protein (Adler-Nissen, 1986). High solubility of protein hydrolysate is due to polar residues of smaller peptide formed by hydrolysis (Sathivel *et al.*, 2005). Polar residues increase the ability to form hydrogen bonds with water, thus, increasing the hydrophilicity (Gbogouri *et al.*, 2004).

#### **Emulsifying properties**

Extensive hydrolysis causes marked loss in emulsifying properties (Gbogouri *et al.*, 2004; Souissi *et al.*, 2007; Foh *et al.*, 2010). This can be seen by significantly lower ( $p < 0.05$ ) emulsion capacity and stability of TAH than CH and LIH (Table 1). A high DH (84.20%) was obtained for hydrolysates produced after pre-treatment with tamarind (TAH). Hydrolysates produced at high DH mainly comprises of short peptides and amino acids (Souissi *et al.*, 2007). Stable emulsion requires high content of larger or more hydrophobic peptides (Mutilangi *et al.*, 1996). Unlike large peptides, short peptides are less efficient in decreasing the interfacial tension because they lack the ability to unfold and reorient at the interface (Gbogouri *et al.*, 2004).

#### **Water and oil holding capacity**

Water holding capacity (WHC) refers to the ability of protein to imbibe water and retain it against a gravitational force within a protein matrix (Fennema, 1996). The result shows that TAH had a significantly ( $p < 0.05$ ) lower WHC than other hydrolysates (Table 1). Fresh mince tilapia hydrolysate and hot water dip tilapia hydrolysate prepared using flavourzyme had a WHC of 2.77 and 2.10 ml/g, respectively (Foh *et al.*, 2010). Oil holding capacity (OHC) decreased with the increased in DH (Table 1). The OHC are within the range of 2.21 to 3.06 ml/g and TAH had the lowest OHC. The digestion of proteins may bring out of the core proteins non polar side chains that join hydrocarbon moieties of oil, thus, causing oil absorption to increase (Lqari *et al.*, 2005). It can be seen that the WHC and OHC of the hydrolysates decreased with increase in DH. The denaturation of protein seems to impair the WHC and OHC of the hydrolysate. Denaturation results in loss of the protein function due to structural change in the protein which might be caused by acidity from the lime and tamarind solution.

### Sensory analysis

The fishy flavor, fishy odor, umami taste, sweetness and bitterness were determined using 15 cm line scale anchored from absent to very strong. The fishy flavor, fishy odor and umami of CH were rated as having between strong and very strong intensity whereas sweetness and bitterness as between weak and moderate (Figure 1). The fishy flavor, fishy odor, umami and bitterness of TAH were rated as having between weak and moderate intensity whereas sweetness as between moderate and strong. This shows that soaking in tamarind juice is an effective pre-treatment in reducing the fishy flavor, fishy odor and bitterness. However, it seems that the umami intensity of the hydrolysate was reduced by the acidity of tamarind juice. TAH had the highest sweetness intensity than CH and LIH which may be due to tamarind which has a tint of sweet taste. Previous studies showed that tilapia fillet pre-treated with 1.5% w/v tamarind juice scored higher for flavor, odor and acceptability than pre-treatment in 1.5% v/v lime juice (Jamilah and Siti Aini, 1997). It is postulated that lime and tamarind juice effectively removed muddy flavor thus increase acceptability of the fish (Jamilah and Siti Aini, 1997).

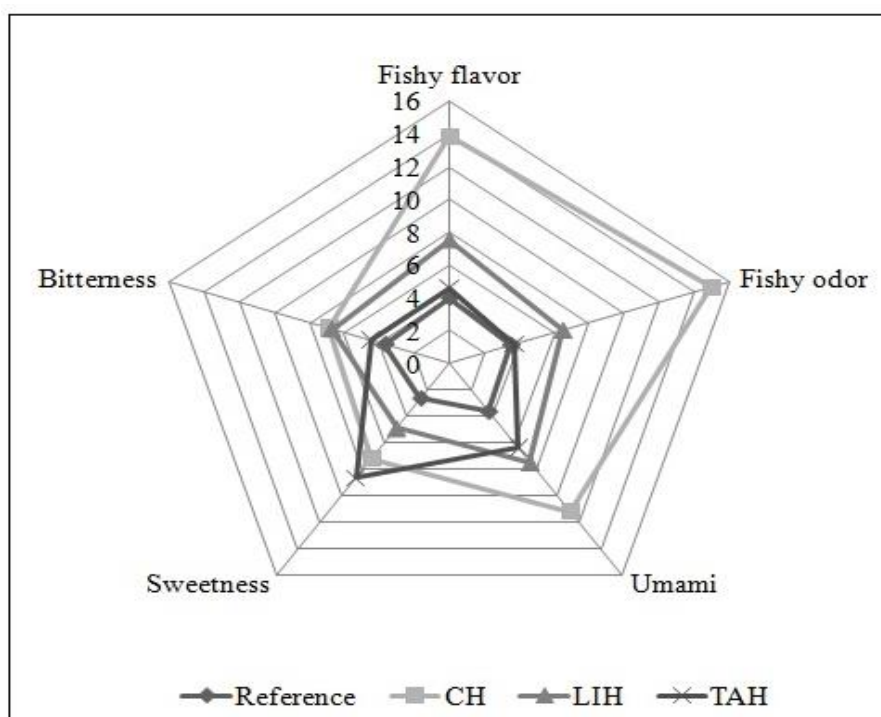


Figure 1. Sensory analysis of silver catfish (*Pangasius sutchi*) hydrolysate prepared after pre-treatment by soaking in water (CH), lime juice (LIH) or tamarind juice (TAH)

### Conclusion

Pre-treatment in tamarind juice (TAH) produced highest DH and yield than other hydrolysates. Besides, TAH had low intensity of fishy odor and flavor that makes it more applicable in food system. Although such pre-treatment impaired the physicochemical properties of the hydrolysate including moisture, protein content and functional properties, the sensory properties were improved.

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