ANTIOXIDANT ACTIVITIES OF PROTEIN HYDROLYSATES FROM RED TILAPIA (*Oreochromis niloticus*) FILLET AS INFLUENCED BY THERMOLYSIN AND ALCALASE

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Abstract – Protein from Red Tilapia (*Oreochromis niloticus*) was hydrolyzed by thermolysin and alcalase under optimum conditions. The hydrolysis was performed for 0, 0.5, 1, 2, 3 and 4 h. Hydrolysates after 2 h incubation with thermolysin and alcalase had achieved degree of hydrolysis of 76.29 % and 63.49 %, respectively. The freeze dried protein hydrolysate was tested for peptide content and characterized with respect to amino acid composition. The result of peptide content showed that the *O. niloticus* hydrolysates obtained was directly proportional increased with different proteolytic enzymes activity. The result of amino acid composition exhibited that alcalase was observed to have a high specificity for Phe, Trp, Tyr, Glu, Leu, Ala, and Lys residues while thermolysin was specifically catalyzes hydrolysis of peptide bonds containing hydrophobic amino acids. Both protein and hydrolysates were tested for anti-oxidant activity by DPPH and ABTS assay. Alcalase may give higher anti-oxidant peptides after 1 hour incubation, but lower than thermolysin hydrolysates after 2 hours by a significant decrease of anti-oxidant activity. Hydrolysates from Red Tilapia may contribute as a health promoting ingredient in functional foods to reduce oxidation stress caused by accumulated free radicals.

Key Words – Oxidative stress, Protein hydrolysates, Proteolytic enzyme

I. INTRODUCTION

Oxidative stress is an imbalance between generation of reactive oxygen species (ROS) and antioxidant defense capacity of the body. The stress may be influenced by many factors include sources from inside cell (normal respiration, exercise, hypoxia, xanthine oxidase and catecholamines) and from outside cell (ultra-violet light, environmental pollutants, pollutants in food, and byproducts of other metabolic processes in organisms) [1,2,3]. Variety types of anti-oxidant compounds have been taken to control free radicals produced by the oxidation process, include enzymatic, non-enzymatic, synthetic chemical and bioactive peptide.

Recently, bioactive peptides were widely studied and have been focused due to increasing demand for natural and safe source of antioxidants. Protein hydrolysates with antioxidant properties, in particular, have become a topic of great interest for the pharmaceutical, health food, as well as the food processing and preservation industries [4,5]. Protein and protein hydrolysates derived from sources like milk, soy, egg, and fish were shown to exhibit antioxidant activity in various muscle foods [5,6,7].

Red Tilapia, *Oreochromis sp.,* is increasing in popularity among producers and contributes approximately 90% of the total Tilapia production in Malaysia. The production of Red Tilapia increased from 8,214 tonnes in 1998 to 20,061 tonnes in 2003, and was targeted to reach at 150,000 tonnes by 2010 [8,9,10]. The *Oreochromis sp.* in general is widely cultured in ponds, cages and tanks as well as in pen culture systems.

The objective of this study is to evaluate the antioxidant ability of protein hydrolysates obtained from Red Tilapia (*O. niloticus*) fillet, hydrolysed by Thermolysin and Alcalase in relation to the degree of hydrolysis, tested with different assays, DPPH radicals scavenging

activity and ABTS radicals scavenging activity.

II. MATERIALS AND METHODS

O. niloticus, 1.0 to 3.0 kg, was obtained from a local supplier at Wet Market, Kajang, Selangor. For enzymatic hydrolysis, the protein was mixed with distilled water with a ratio of 2:100 (w/v) and was adjusted to optimal pH and temperature for thermolysin and alcalase (pH 7.4; 37 ˚C). Ratio of enzyme to sample was 1:100 (v/w). The hydrolysis was performed for 0 to 4 hour in a shaking water-bath incubator. Enzyme reaction was inactivated with temperature at 90 °C for 10 min. The samples were then centrifuged at 3000x*g* for 20 min and the soluble hydrolysates were freeze-dried.

Degree of hydrolysis (DH) of protein hydrolysates obtained was analyzed according to percent of trichloroacetic acid (TCA) method as described by Hoyle and Merritt [11]. The supernatant was analyzed for soluble nitrogen using kjeldahl method (Kjeltec 2100, Foss, Denmark) [12].

Peptide content was measured by Church et al. [13] method with modification using o-phthaldialdehyde (OPA) spectrophotometer (Model UV-160A). Leucine was used as a standard.

The amino acid composition was examined by a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Fluorescence and AccQ Tag column (3.9 x 150 mm).

DPPH radical-scavenging activity and ABTS radical-scavenging activity of both enzymatic hydrolysates were determined by DPPH assay and ABTS assay, as described by Binsan et al. [14].

in means between samples were determined at 5% confidence level ($P < 0.05$).

III. RESULTS AND DISCUSSION

Hydrolysis of *O. niloticus* protein was characterized by a high rate of hydrolysis

All data collected was analyzed using the analysis of variance (ANOVA) and Duncan's multiple range tests. The significant difference with alcalase enzyme. The DH of protein hydrolysates by thermolysin and alcalase after 2 h incubation was 76.29 % and 63.49 %, respectively.

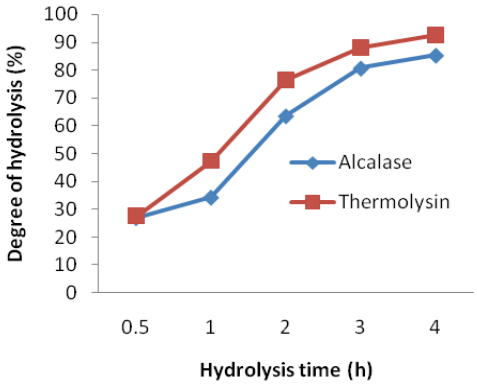

Figure 1: Degree of hydrolysis of *O. niloticus* protein with thermolysin and alcalase for 0.5 to 4 h.

The quantification of peptide content was directly proportional with the increasing of degree of hydrolysis as shown in Table 1. The presence of low molecular weight in protein hydrolysates obtained may result in production of bioactive peptides with potent antioxidant properties.

Table 1: Effect of hydrolysis time and different enzymes (Thermolysin and Alcalase) on degree of hydrolysis and peptide content of *O. niloticus* protein hydrolysate.

Time (h)	Thermolysin		Alcalase	
	DH (%)	Peptide content (ug/ml)	DH (%)	Peptide content (ug/ml)
0.5	27.41	0.18	26.85	0.14
1.0	47.30	0.29	34.19	0.21
2.0	76.29	0.46	63.49	0.38
3.0	88.01	0.53	80.66	0.49
4.0	92.58	0.56	85.30	0.51

during the first 1-2 h (Figure 1). The rate of hydrolysis with thermolysin was higher than
In this sudy, all 20 amino acids were detected in protein and hydrolysates of sample and contain abundants of Gly, Ala, Asp, Glu, Lys and Leu in residues or peptide sequences. Alcalase was observed to have a high

specificity for Phe, Trp, Tyr, Glu, Leu, Ala, and Lys residues. Thermolysin was specifically catalyzes hydrolysis of peptide bonds containing hydrophobic amino acids. Hydrophobic amino acid residues like Val, Leu at N-terminus end and Pro, His or Tyr in the sequence of peptides, may involve in antioxidative activities. Some amino acids such as Gly, Ala, and Met are also believed to enhance the activities of antioxidant peptides [15,16]. Enzymatic hydrolysates at 1 and 2 hours were chosen for further analyzed in determination of antioxidant activity.

DPPH radical-scavenging activity is generally used to determine hydrogen-donating ability of protein hydrolysates. As shown in Table 2, hydrolysates by alcalase resulted in higher antioxidant activity than hydrolysates by thermolysin enzyme. The antioxidant activity was higher at 1 hour enzymatic hydrolysates than 2 hour for thermolysin and alcalase with 79 and 112 µg/ml TE, respectively. Both enzymatic hydrolysates were further test with ABTS assay.

Table 2: Effect of hydrolysis time and different enzymes (Thermolysin and Alcalase) on anti-oxidant activity of *O. niloticus* protein hydrolysate with DPPH assay expressed as µg/ml of Trolox equivalent (TE).

Samples	Hydrolysis time (h)	DPPH activity (µg/ml)	
		Thermolysin	Alcalase
Actual protein		12[a]	
Peptides	1	79[a]	112[a]
	2	70[a]	105[b]

[ab]Different lowercase letters indicate significant differences ($P < 0.05$) between samples.

ABTS assay is a measure of antioxidant activity which might include a proportion of biologically inactive antioxidants, in the mean of chain-breaking antioxidants. The present study shows hydrolysates by alcalase give low significant difference of antioxidant activity with hydrolysates by thermolysin in the 1 hour incubation but lower after 2 hour incubation compared to hydrolysates by thermolysin, with 80 and 95 µg/ml TE, respectively, as shown in Table 3. It has been reported that the highest DPPH radical scavenging activity in Sardinell (*Sardinella aurita*) was 150 µg/ml [10], which are almost similar with the anti-oxidant value with the hydrolysate from Red Tilapia when hydrolysis with alcalase in the present study. According to Nalinanon et al. [17], at 30% DH showed activities of 151 µmol TE/g protein, for ABTS assay, which are also close with the present value for both enzymatic hydrolysates.

Table 3: Effect of hydrolysis time and different enzymes (Thermolysin and Alcalase) on anti-oxidant activity of *O. niloticus* protein hydrolysate with ABTS assay expressed as µg/ml of Trolox equivalent (TE).

Samples	Hydrolysis time (h)	ABTS activity (µg/ml)	
		Thermolysin	Alcalase
Actual protein		45[b]	
Peptides	1	145[a]	144[b]
	2	95[a]	80[b]

[ab]Different lowercase letters indicate significant differences ($P < 0.05$) between samples.

IV. CONCLUSION

Anti-oxidant peptides from protein hydrolysates of Red Tilapia (*O. niloticus*) hydrolysed by thermolysin and alcalase have been identified. Hydrolysates with alcalase was higher than thermolysin in screening assay of anti-oxidant acitivity. At 2 hours incubation, both hydrolysates by alcalase and thermolysin show a decrease of anti-oxidant activity. It was concluded that alcalase may give higher anti-oxidant peptides after 1 hour incubation, but lower than thermolysin hydrolysates after 2 hours hydrolysis. It is recommended that potent anti-oxidant peptides extracted from Red Tilapia *O. niloticus* to be further study to characterized and synthesis the anti-oxidant peptides as a health promoting ingredient in functional foods to reduce oxidation stress caused by accumulated free radicals.

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