Physicochemical and Functional Properties of Mixed Fishes Hydrolysates Obtained Enzymaticly from *Apogon albimaculosus, Platycephalidae cymbacephalus and Cynoglossus lingua*

Afan Bagus Mananda[1], Yuli Witono[1], Iwan Taruna[1] and Woo-Won Kang[2]

[1] Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Jember, Indonesia, Email: ylwitono@yahoo.com

[2] Department of Food Science & Nutrition, College of Science & Engineering, Kyung Pook National University, Korea, Email: wwkang@knu.ac.kr

ABSTRACT

Fish Protein Hydrolysates (FPH) have a good nutritional properties and used widely in the food industry for various purpose, protein supplement, surimi production, beverage stabilizer, and flavor enhancers. The aim of this research was to evaluate how the enzyme and activator influence the yield, protein solubility, maillard, level of rancidity, and the amino acid profiles of mixed fishes protein hydrolysates. A series of hydrolysis trials have been carried out using different ratio of 'biduri' protease : papain (30:70; 50:50; and 70:30) and concentration of sistein as activator (0; 1; and 6%). Higher concentration of biduri and higher concentration of sistein gave increased the maillard value and decrease the level of rancidity and protein solubility. Among the treatment, ratio biduri protease and papain 70:30, and sistein 0,6% demonstrated the best treatment based on the rancidity and protein solubility. FPH have potential to enhance product stability by preventing oxidative deterioration. The DPPH scavenge activity showed that antioxidative activity of mixed FPH 6.98% ±1.59 could be due to the ability to scavenge lipid radicals. This study also showed that mixed FPH was potential as flavour enhancer. The total content of bioactive peptides were 1200.34 mg/100 g and glutamate 41.32 mg/100 g could make the hydrolysates useful for incorporation as flavour enhancer in food.

Key words: *mixed fishes protein hydrolysates, biduri protease, papain, sistein, protein solubility, flavour enhancer*

INTRODUCTION

Bibisan (*Apogon albimaculoses*), Baji-baji (*Platycephalidae cymbacephallus*), and Lidah (*Cynoglossus lingua*) are populer freshwater fishes in Madura island, Indonesia. They have a low price because of abundant and only use as food. Thus, no information has been reported on the production of enzymatic protein hydrolysis from them.

Enzymatic proteolysis and solubilization of proteins from various sources has been studied extensively and described by several different authors over the last 60 years (Aspmo *et al.,* 2005; Petreus *et al.,* 2011). Addition of proteolytic enzymes could make a hydrolytic

process more controllable. Protease produced from Biduri (*Calotropis gigantea*) has been proven to be one of the enzyme used in the preparation of fish protein hydrolysates (Witono, 2011). Biduri is one kind of wild shrub with 0.5-3 meters height that grows in place with dry periods such as Indonesia.

Enzymatic hydrolysis is one of the methods for recovery of valuable components from fish. Fish protein hydrolysates (FPH) have good solubility over a wide range of ionic strength and pH and usually tolerate strong heat without precipitating. FPH have good functional properties and can contribute to water holding, texture, gelling, whipping and emulsification properties when added to food (Kristinsson, 2007). Some studies have shown that FPH can contribute to increased water holding capacity in food formulations (Wasswa *et al.*, 2008); and addition of FPH from salmon reduced water loss after freezing (Kristinsson and Rasco, 2000). Fish protein hydrolysates (FPH) have good foaming and emulsifying properties, thus may be used as emulsifying and emulsion stabilizing ingredients in a variety of products as well as aid in the formation and stabilization of foam-based products. Because the size of the peptides is very important for interfacial/surface activity of FPH, the degree of hydrolysis is important (Jeon *et al*., 2000). Several reports have suggested that there is an optimum molecular size or chain length for peptides to provide good foaming and emulsifying properties, and that limited hydrolysis resulting in larger peptides generally leads to improved emulsification and foaming properties, while extensive hydrolysis resulting in small peptides reduce these properties (Kristinsson dan Rasco, 2000). In addition, except for the deficit of a few amino acids, hydrolysates have a high nutritional value (Dauksas *et al*., 2005).

Several studies have indicated that peptides derived from fish proteins have antioxidative properties in different oxidative systems (Kristinsson, 2007). The antioxidant activity of proteins and peptides can be the result of specific scavenging of radicals formed during peroxidation, scavenging of oxygen containing compounds, or metal-chelating ability (Gutierresz *et al*., 2003). Production of fish protein hydrolysates with antioxidant properties will enable production of protein enriched and oxidative stable seafood the sequences essential for biological activity.

The aim of the present study was to evaluate how the combination of biduri protease and papain, added by activator influence the yield, protein solubility, maillard, level of rancidity, antioxidative activity and the amino acid profiles of mixed FPH.

MATERIALS AND METHOD

Bibisan (*Apogon albimaculoses*), Baji-baji (*Platycephalidae cymbacephallus*), and Lidah (*Cynoglossus lingua*) were obtained from Madura Island, East Java, Indonesia. Fresh fish was filleted and the fish meat was stored in polyethylane bag at 4^{o}C until used for FPH production. The proteolytic enzyme used protease from biduri and papain that produced by sentrifugated method.

Production of Fish Protein Hydrolysates (FPH)

The samples of mixed meat fishes were partly thawed at room temperature and mixed with distillate water (1:2) and blended for 2-3 minutes. The homogenate sampless were adjusted to pH 7.00 with buffer addition and added by activator (sistein). The hydrolysis process was done in waterbath (Memmert, Germany) set up at 55^{o}C. The enzymatic hydrolysis

was started by added 1,5% of the combination of biduri protease:papain at different concentration (30:70; 50:50; 70:30) and different concentration of sistein as catalisator (0; 0,1; and 0,6%). The enzyme was inactivated by heating at 100 ^{o}C for 10 minutes. The mixtured was then drying by oven at 40^{o}C during 18 hours. The sampel will be mashed until 60 mesh and the be analyzed.

Functional Properties Analysis

Solubility and nitrogen solubility index were calculated to determine the solubility of protein hydrolysates, following the prosedure of Morr (1985). Maillard value was determined according to the method proposed by Subagio *et al*. (2002), and the rancidity by Hofmann *et al*. (1999) method. The antioxidative activity of FPH determined using an indirect spectrophotometric assay, the DPPH method as described by Thiansilakul *et al*. (2007).

Amino Acid Analysis

Sample preparation and analysis of free and total amino acids were carries out according to the methods of Cha and Cadwallader (1998) and Ha *et al*. (2001), respectively, the total and free amino acids were quantitatively and analyzed using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, USA).

RESULTS AND DISCUSSION

Protein Solubility

Solubility is one of the most important FPH functional properties. Good solubility of proteins is required in many functional applications, including emulsions, foams, and gels. Solubility of FPH using different concentration of enzyme and sistein are presented in figure 1.

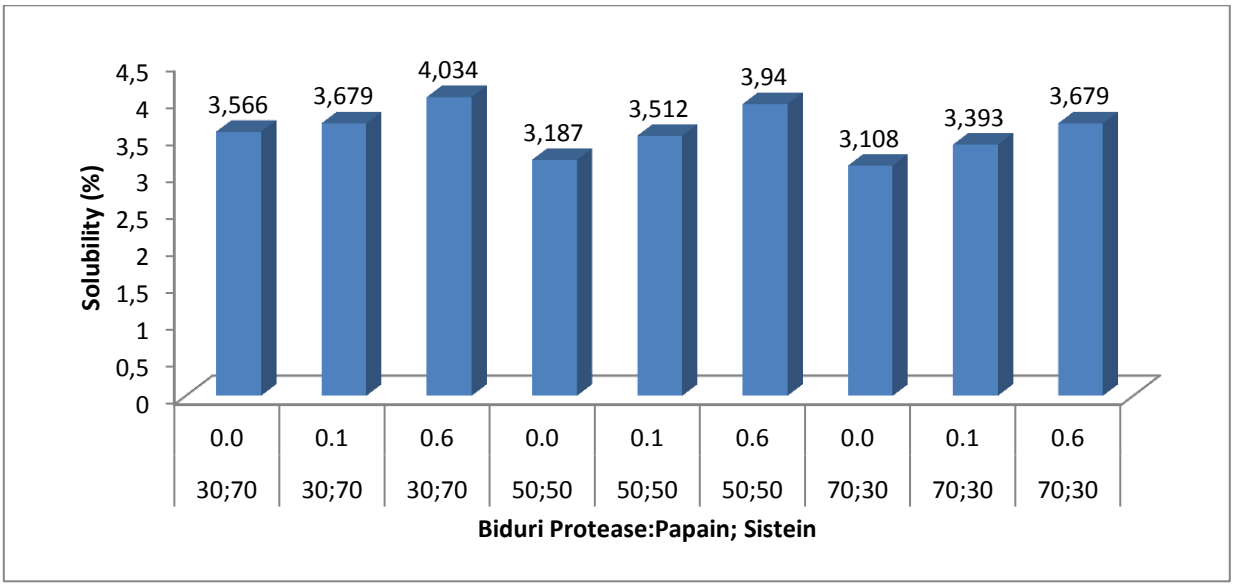


Figure 1. Solubility of FPH in different concentration enzyme and sistein as activator

Enzymatic hydrolysis of fish proteins generates a mixture of free amino acids, di-, tri- and oligopeptides, increasing the number of polar groups, which promotes the interaction with water, i.e. increased solubility, which also plays an important role in other functional properties, like foaming and emulsifying capacity (Kristinsson and Rasco, 2000).

Maillard

Maillard raction caused non-enzymatic browning. The results indicated that the higher concentration of biduri protease and sistein caused higher absorbance in maillard analysis (Figure 2).

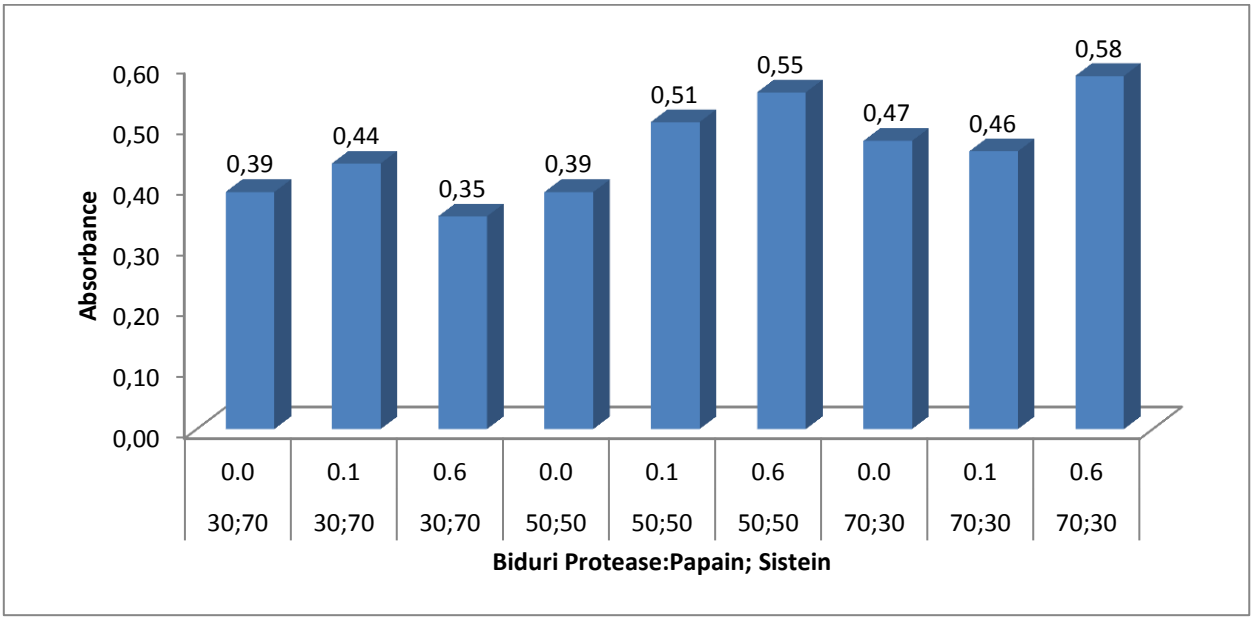
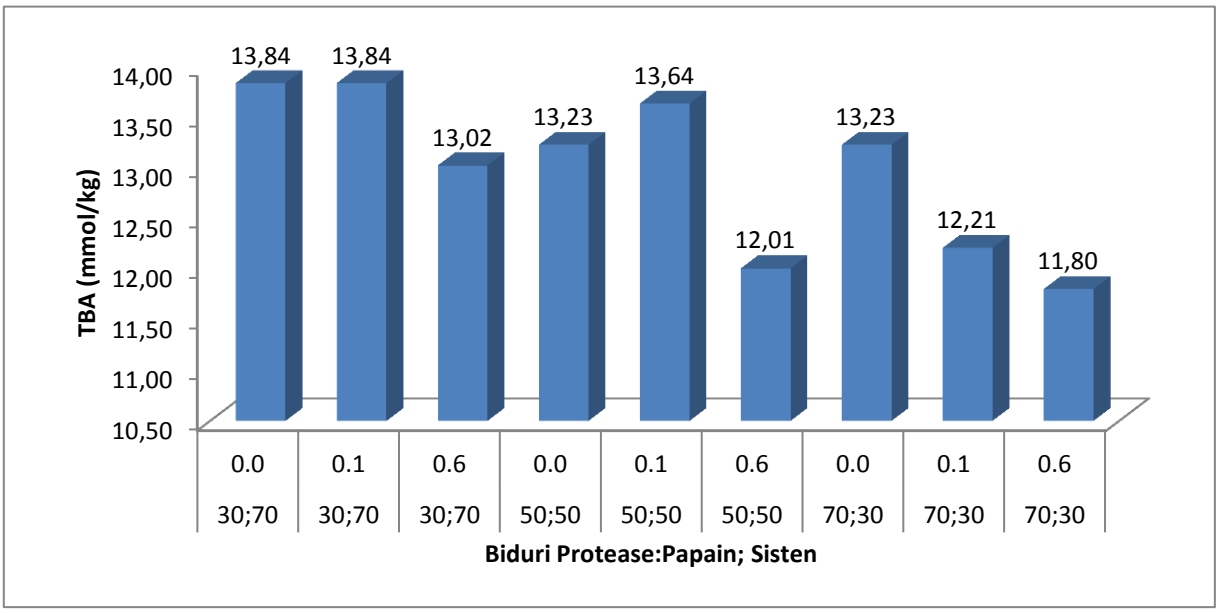


Figure 2. Maillard absorbance in different concentration enzyme and sistein

Certain protein hydrolysates have previously been reported to be antioxidative. However the antioxidant effect of protein hydrolysates was conciderably improved when reacting with glucose. Lingnert and Eriksson investigated the ability of peptides to form antioxidative maillard reaction product (MRP) and conclude that potent antioxidants were able to form when reacting peptides with sugars as antioxidative effect is dependent not only on which amino acids constitute the peptide but also on their sequence.

Rancidity

Rancidity was caused by lipid peroxidation that more decrease with the higher of biduri protease concentration in FPH production. The results of rancidity described in figure 3.



Figuri 3. Rancidity of FPH in TBA (thiobarbituric acid) value (mmol/kg) in different concentration enzyme and sistein

Lipid peroxidation is of great concern to the food industry and consumers because it leads to the development of undesirable flavors, odors and impair the nutritional value of foods. Use of synthetic antioxidants is under strict regulation because of the potential health hazards caused by such compounds (Park *et al*. 2001). Antioxidants derived from food ingredients have the potential to minimize the oxidation of lipids during processing and storage of foods. Fish protein hydrolysates from different species have been found to retard lipid oxidation and are feasible to use as natural antioxidants in foods and biological systems. Peptides isolated from fish protein hydrolysates have been reported to have antioxidant activity (Najafian and Babji 2011; Ngo *et al*. 2011).

Antioxidant Activity

The analysis of the best treatment indicated that FPH from mixed fishes have antioxidant activity 6,98% ±1,59 %. In recent years, several studies have described the antioxidant activity of protein hydrolysates from fish sources, like yellowfin sole (Jun *et al*., 2004), cobia skin (Yang *et al*., 2008), tuna liver (Je *et al*., 2009), sardinelle by-products (Bougatef *et al*. 2010) and backbone from Baltic cod (Zelechowska *et al*., 2010).

Many authors referred that different amino acid residues may have their specific roles in peptides antioxidant activity, which can be by chelation of transition metals and/or scavenging free radicals. In a review presented by Chalamaiah *et al*. (2012), it is enhanced the importance of hydrophobic amino acids and one or more residues of histidine, proline, methionine, cysteine, tyrosine, tryptophan, valine, leucine and phenylalanine in the antioxidant activity of peptides. The presence of hydrophobic sequences in the peptides allows them to interact with lipid molecules and could scavenge by donating protons to lipid derived radicals.

The aromatic amino acids, tyrosine and phenylalanine, as well as histidine, methionine and cysteine are indicated as direct radical scavengers. Tyrosine's antioxidant activity is due to the capability of phenolic groups to serve as hydrogen donors; the imidazole group in histidine has the proton-donation ability; methionine is prone to oxidation of the methionine sulfoxide; cysteine donates the sulfur hydrogen. The imidazole group in histidine has also been reported has metal chelator. Acidic and basic amino acids may also play an important role in Fe2+ and Cu2+chelation (Carrasco-Castilla *et al.*, 2012).

Amino Acids Composition of Hydrolysate

Amino acids composition of the beluga visceral protein hydrolysates at optimum conditions are presented in Table 1.

Table 1. The amino acid composition of protein hydrolysate of mixed fishes (mg/100g)

Amino Acids	Quantity
Taurine	21,4096
Urea	62,4585
hydroxyproline	12,9704
Threonine	7,5911
Asparagine	46,9490
glutamic acid	41,3233
a-aminoadipic acid	42,6354
Proline	46,9372
Glycine	15,9113
Alanine	6,6515
Citrulline	93,6190
a-aminobutyric acid	83,6453
valine	69,1407
methionine	29,6639
cystathionine	134,9617
isoleucine	66,7946
tyrosine	133,9285
g-aminobutyric acid	88,0538
ethanolamine	6,6620
ammonium chloride	6,2208
hydroxylysine	10,3009
ornithine	77,3905
lysine	14,2250
anserine	15,5916
carnosine	46,2556
arginine	18,8432
	1200,1344

CONCLUSION

The protein hydrolysate derived mixedfishes using biduri protease and papain enzyme may potentially serve as a good source of protein. Fish protein hydrolysates by biduri protease:papain 70:30, and sistein 0,6% demonstrated the best treatment based on the rancidity and protein solubility. This treatment showed that antioxidative activity 6,98% ±1,59 could be due to the ability to scavenge lipid radicals and content of bioactive peptides were 1200,34 mg/100g) and glutamat 41,32 mg/100 g could make the hydrolysates useful for incorporation as flavour enhancer in food.

ACKNOWLEDGEMENT

We thank Research and Community Service Directorate of Indonesia High Education Directorate for financially supporting this research through National Strategic Research Grant Program of Budget Year 2013.

REFERENCES

Bougatef, A, Nedjar-Arroume, N, Manni, L, Ravallec, R, Barkia, A, Guillochon, D, Nasri, M (2010). Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products proteins. *Food Chemistry*, 118: 559–565.

Carrasco-Castilla, J, Hernández-Álvarez, AJ, Jiménez-Martínez, C, Alaiz, M, Girón-Calle, Vioque, J, Ortiz-Dávila, G (2012). Antioxidant and metal chelating activities of peptides fractions from phaseolin and bean protein hydrolysates. *Food Chemistry*. 135: 1789 – 1795.

Cha YJ and Cadwalker KR, 1998. Aroma-active compounds in skipjack tuna sauce. J. Agric Food Chem 46:1123 – 1128.

Chalamaiah, M, Dinesh Kumar, B, Hemalatha, R, Jyothirmayi, T (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications – A review. *Food Chemistry*, 135: 3020 – 3038.

components. Evaluation of their antioxidant activity in a liposomal model system. Life Sciences 2003;72(21):2337–60.

Dauksas E, Falch E, Slizyte R, Rustad T. Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (Gadus morhua) by-products. Process Biochemistry 2005;40(8):2659–70.

Gutierrez ME, Garcia AF, de Madariaga MA, Sagrista ML, Casado FJ, Mora M. Interaction of tocopherols and phenolic compounds with membrane lipid components. Evaluation of their antioxidant activity in a liposomal model system. Life Sciences 2003;72(21):2337–60.

Ha JH, Kim PH, Heu MS, Cho ML, SIm HD, Kim JS, and Kim IS. 2001. Comparison of regional variation in food component of top shell, Omphalius pfeifferi capenteri. J Korean Fish Soc 34: 384-388.

Hofmann T, Bors W, dan Stettmaier K, 1999. Studies on Radical Intermidiates in the Early Stage of the Nonenzymatic Browning Reaction of Carbohydrates and Amino Acids, *J. Agric. Food Chem.* 47: 379–390.

Je, JY, Qian, ZJ, Byun HG, Kim SK (2007). Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. *Process Biochemistry*, 42: 840 - 846.

Jeon YJ, Byun HG, Kim SK. Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochemistry 2000; 35(5):471–8.

Jun, S, Park, P, Jung, W, Kim, S (2004). Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. *European Food Research and Technology*, 219: 20–26.

Kristinsson, H.G, and Rasco, B.A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition 2000;40(1):43–81.

Kristinsson, H.G. (2007). Aquatic food protein hydrolysates. In: Shahidi F, editor. Maximising the value ofmarine by-products.Woodhead Publishing Ltd.; 2007.

Morr, C.V. (1985). Composition physicochemical and functional properties of reference whey protein concentrate. Journal of Food Science, 50:1406-1411.

Najafian, L. and Babji, A.S. 2011. A review of fish derived antioxidant and antimicrobial peptides: Their production assessment and applications. Peptides *33*, 178–185.

Ngo, D.H.,WIJESEKARA, I., VO, T.S., TA, Q.V. and KIM, S.K. 2011. Marine food derived functional ingredients as potential antioxidants in the food industry: An overview. Food Res. Int. *44*, 523–529.

Subagio A, Hartanti S, Windrati WS, Unus, Fauzi M, dan Herry B, 2002. Characteristics of protein hydrolysate from tempe, *Jurnal Teknologi & Industri Pangan*, 8: 204–210.

Thiansilakul Y, Benjakul S, Shahidi F. Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. Journal of Food Biochemistry 2007;31(2):266–87.

Wasswa J, Tang J, Gu XH. Functional properties of Grass carp (Ctenopharyngodon idella) Nile perch (Lates niloticus) and Nile tilapia (Oreochromis niloticus) skin hydrolysates. International Journal of Food Properties 2008;11(2): 339–50.

Zelechowska, E, Sadowska, M, Turk, M (2010). Isolation and some properties of collagen from the backbone of Baltic cod (*Gadus morhua). Food Hydrocolloids*, 24: 325 - 329.