# Protease Inhibitory Activity and Protein Analysis of Catfish (Pangasius hypopthalmus) and Swamp Eel (Monopterus albus) Blood Plasma

*by* Ace Baehaki

**Submission date:** 02-Jul-2019 12:09PM (UTC+0700)
**Submission ID:** 1148620117
**File name:** Protease_Inhibitory_Activity_and_Protein_Analysis_of_Catfish.pdf (475.71K)
**Word count:** 4149
**Character count:** 21429

Pertanika J. Trop. Agric. Sc. 42 (1): 155 - 164 (2019)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

# Protease Inhibitory Activity and Protein Analysis of Catfish (*Pangasius hypopthalmus*) and Swamp Eel (*Monopterus albus*) Blood Plasma

**Rodiana Nopianti[1,2]*, Herpandi[1], Ace Baehaki[1], Rinto[1], Sherly Ridhowati[1] and Maggy Thenawidjaja Suhartono[2]**

[1]*Department of Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, 30662, Indonesia*

[2]*Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural Institute, Dramaga Bogor 16002, Indonesia*

## ABSTRACT

Protease inhibitors can prevent protein from degradation caused by protease activity. Blood plasma contains a variety of protease inhibitors. The objective of this study was to investigate the potential use of crude blood plasma from catfish (*Pangasius hypopthalmus*) and swamp eel (*Monopterus albus*) as protease inhibitors. The parameters observed were moisture content, protein content, ash content, inhibitory activity to trypsin and papain enzymes and protein profile of blood plasma. The inhibitory activity increased as the volume of blood plasma increased (25 µL, 50 µL, 75 µL, 100 µL). The inhibitory activity of blood plasma from catfish was 7.66-50.73% to trypsin enzyme and 20.34-83.05% to papain enzyme while the inhibitory activity of blood plasma from swamp eel was 9.49-46.35% to trypsin enzyme and 28.81-64.41% to papain enzyme. The highest inhibitory activity was demonstrated by swamp eel blood plasma with molecular weight between 19.84-174.14 kDa (13 proteins). Protein content of blood plasma from swamp eel (11.92%) was lower than catfish (16.63%) but resulted higher enzyme inhibitory activity. Further research can be conducted to carry out purification steps on blood plasma that are expected to show better inhibitory activity.

*Keywords:* Blood plasma, catfish, protease inhibitor, swamp eel

ARTICLE INFO

*Article history:*
Received: 21 March 2018
Accepted: 26 September 2018
Published: 26 February 2019

*E-mail addresses:*
nopi_81@yahoo.com (Rodiana Nopianti)
herpandinapis@gmail.com (Herpandi)
ace76_none@yahoo.com (Ace Baehaki)
rinto.unsri@gmail.com (Rinto)
sherlyridhowati@gmail.com (Sherly Ridhowati)
mthenawidjaya@yahoo.com (Maggy Thenawidjaja Suhartono)
* Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

## INTRODUCTION

Fish is a commonly used food around the world and is classified as perishable food. One of the factors that affect the rapid change in fish quality is the high protein content. In general, protein content in fish is 15-20%, but protein content lower than 15% and higher than 28% is also found in some fish species (Murray & Burt, 1983). Protease enzymes act on fish protein to bring about its deterioration in its quality.

The decline in quality after the post mortem phase is one of the most unfavourable changes in fish muscle. During post mortem, degradation of muscle proteins contributes to the rapid softening of flesh. The protein proteolysis can be attributed to endogenous protease activity (Chéret et al., 2007). Two characterized proteolytics are known to hydrolyze protein during post mortem storage of meat and fish muscle: calpains and cathepsins (Jiang, 2000; Ouali, 1992). Cathepsin L is one of the most important protease enzymes in the softening process in fish meat, and in surimi processing it is a problem because washing cannot eliminate it. The presence of cathepsin enzyme in surimi or fish mince results in a decrease in gel strength with a brittle and non-elastic gel at temperature around 60°C (Rawdkuen et al., 2007b). Ho et al. (2000) reported that cathepsin L and and L-like in mackerel surimi had Myosin Heavy Chain (MHC)-degrading ability which consequently caused gel softening during setting at 40-45°C. In addition, texture softening also occurs in grass carp fillets caused by Cathepsin B and L as the major endogenous enzymes that leads to proteolytic degradation (Ge et al., 2014).

One way to inhibit proteolytic degradation by cathepsin enzymes is by using blood plasma. Blood plasma contains a variety of protease inhibitors, including α2-macroglobulin which is a protease inhibitor of some protease classes with its mechanism of action baits and traps (Barret, 1981). Many studies have been conducted using plasma to inhibit the work of cathepsin enzymes, including cow blood plasma (Kang & Lanier, 1999; Marquez-Alvarez et al., 2015), pig blood plasma (Benjakul et al., 2001; Benjakul & Visessanguan, 2000), chicken blood plasma (Rawdkuen et al., 2007a) and salmon blood plasma (Fowler & Park, 2015). However, the utilization of blood plasma from cattle and chickens is limited due to mad cow disease infection as well as avian influenza in poultry.

Blood plasma comprises 46-63% of total blood volume, with moisture content being 92% (Martini, 2005). According to the Food and Agriculture Organization (Nomura, 2007), the global fish catch and aquaculture amounted to 106 million tons in 2004, and since blood is about 7% of body weight (Philips & Williams, 2011), this would amount to 7 million tons. Fish blood is generally not utilized but has the potential to be collected and processed to produce value-added foods based on its nutritional value and functional properties (Lynch et al., 2017). Fish blood can be extracted for application in the food, biomedical and pharmaceutical sectors (Lafarga et al., 2015; Lafarga et al., 2016; Mullen et al., 2015).

The objective of this study was to investigate the ability of blood plasma from two freshwater tropical fish (catfish and swamp eel) to function as protease inhibitor towards the enzymes trypsin and papain.

## MATERIAL AND METHODS

### Materials

Live catfish (*Pangasius hypopthalmus*) (size 2-4/kg) and swamp eel (*Monopterus albus*) (size 10-15/kg) were obtained from Indralaya traditional market, South Sumatera, Indonesia. Trypsin (from bovine pancreas), papain (from papaya latex), $N_{\alpha}$-Benzoyl-$_{L}$-arginine-$_{DL}$ β-naphthylamide (BANA) and $N_{\alpha}$-Benzoyl-$_{L}$-arginine 4-nitroanilide hydrochloride (BAPNA) were purchased from Sigma Aldrich (USA).

### Collecting of Blood Plasma

The collecting of crude blood plasma was according to methods described in Fowler and Park (2015). Whole blood was collected from bleeding fish into bottles containing EDTA (Ethylenediaminetetraacetic acid), and then centrifuged for 15 min at 1500 g at 4°C. The supernatant was regarded as plasma and kept in freezer until it was used.

### Proximate Analysis

**Protein Content (Bradford, 1976).** The protein analysis followed the method of Bradford (1976) and the Bovine Serum Albumin (BSA) was used as the standard.

***Preparation of Bradford Reagent Solution.*** A 25 mL aliquot of ethanol (95%) was mixed with 5 mg of Commassie Brilliant Blue G250. The solution was then added to 50 mL $H_3PO_3$ 85% and homogenized. The mixture was adjusted with distilled water until it reached a volume of 100 mL and kept at 4°C before use.

***Preparation of Standard Solution.*** The preparation of standard solutions for protein analysis is given in Table 1. Ten mg of BSA was added to 10 mL of distilled water and then stirred with a magnetic stirrer to give a homogeneous solution. Specific volumes of BSA (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL) were added to different volume of distilled water (0.9, 0.8, 0.6, 0.4, 0.2 and 0 mL) respectively, and then 5 mL of Bradford solution was added to each mixture. The mixtures were allowed to react for 30 minutes at room temperature. The absorbance was read at 595 nm.

Table 1
*The concentration of standard solution (Bovine Serum Albumin)*

| BSA (mL) | Distilled water (mL) | Concentration (mg/mL) |
|---|---|---|
| 0.1 | 0.9 | 0.1 |
| 0.2 | 0.8 | 0.2 |
| 0.4 | 0.6 | 0.4 |
| 0.6 | 0.4 | 0.6 |
| 0.8 | 0.2 | 0.8 |
| 1 | 0 | 1 |

***Preparation of Blank.*** One mL of distilled water was mixed with 5 mL of Bradford solution which was then homogenized and allowed to react for 30 minutes at room temperature. The absorbance was read at 595 nm.

***Sample Assay.*** A 1 mL of sample plasma was mixed with 9 mL of distilled water. A 0.5 mL of the mixture was then added to 1.5 mL of Bradford reagent. The solution was allowed to react at room temperature for 30 minutes. The absorbance was read at 595 nm. The concentration of protein was determined by the following equation:

ax+b
y = absorbance of sample
a = slope
b = intercept
x = protein concentration of sample

**Moisture Content.** The moisture content was determined using the evaporation principle until the sample reached a constant weight at 105°C (Association of Official Analytical Chemists [AOAC], 2005). The blood plasma sample used was 1 mL.

**Ash Content.** The ash content analysis was determined by the combustion of organic compounds at 550°C (AOAC, 2005). The blood plasma sample used was 1 mL.

**Trypsin Inhibition Assay.** Trypsin inhibition was determined according to the methods of Fowler and Park (2015). Four different blood plasma volumes ((25, 50, 75, 100 µL) (Table 2) were diluted with distilled water and adjusted to 0.2 mL. 150 µL of the inhibitor solution was added to 300 µL of trypsin enzyme (20 µg/mL) and 150 µL of distilled water and pre-incubated at 37°C for 10 min. 750 µL of 0.4 mg/mL BAPNA in 50 mM tris-HCl buffer (pH 8.2) containing 20 mM $CaCl_2$ and pre-warmed to 37°C was than added, and the reaction mixture was incubated for 37°C for 10 minutes. The reaction was stopped by adding 150 µL of 30% acetic acid (v/v). Absorbance was read at 410 nm and inhibitory activity was expressed as percent decrease in $OD_{410}$ compared to the control.

**Papain Inhibition Assay.** Papain inhibition was determined according to the method of Fowler and Park (2015). Four different blood plasma volumes ((25, 50, 75, 100 µL) (Table 2) were diluted with distilled water and

Table 2
*Volume of catfish and swamp eel blood plasma sample for trypsin and papain inhibition assay*

| Sample | Papain Assay | | | Trypsin Assay | | |
|---|---|---|---|---|---|---|
| | Plasma Volume (µL) | Protein Concentration (mg/mL) | Plasma Concentration (mg/mL) | Plasma Volume (µL) | Protein Concentration (mg/mL) | Plasma Concentration (mg/mL) |
| Catfish Plasma | 25 | 0.21 | 1.25 | 25 | 2.08 | 12.5 |
| | 50 | 0.42 | 2.5 | 50 | 4.16 | 25 |
| | 75 | 0.62 | 3.75 | 75 | 6.24 | 37.5 |
| | 100 | 0.83 | 5 | 100 | 8.32 | 50 |
| Swamp eel Plasma | 25 | 0.15 | 1.25 | 25 | 1.49 | 12.5 |
| | 50 | 0.30 | 2.5 | 50 | 2.98 | 25 |
| | 75 | 0.45 | 3.75 | 75 | 4.47 | 37.5 |
| | 100 | 0.60 | 5 | 100 | 5.96 | 50 |

adjusted to 2 mL. 2 mL of 0.25 M sodium phosphate buffer (pH 6.0) containing 2.5 mM EDTA and 25 mM β-mercaptoethanol (βME) was added to 0.1 mL of papain solution (100 µg/mL) containing 25 mM sodium phosphate buffer (pH 7.0) and 2 mL of inhibitor solution. After preincubation at 37°C for 5min, 0.2 mL of 2 mM BANA was added to initiate the reaction. After 10 min of incubation, 1 mL of cold 2% HCl in ethanol was added to stop the reaction. 1 mL of 0.06% ρ-dimethylamino-cinnamaldehyde dye was then added to show the colour of the final solution. Absorbance was read at 540 nm and the inhibitory activity was expressed as the percent decrease in $OD_{540}$ compared to the control.

### Molecular Weight of Inhibitor

The SDS-PAGE procedure was followed according to the method described by Laemmli (1970). A 12% resolving gel and a 3.5% stacking gel were used. The sample buffer consisted of 188 mM M Tris-HCl pH 6.8, 15% β-mercaptoethanol, 3% SDS, 0.01% bromophenol blue, and 30% glycerol in deionized water. Sample preparation was conducted by mixing protein samples with loading buffer in a ratio 1:1 and heating the mixture to 95°C for 10 min to denature the protein samples. An SDS-PAGE broad-range molecular weight standard (6.5 to 200 kDa) (Bio-Rad, Alfred Nobel Drive, Hercules, CA, U.S.A) was used. The running buffer consisted of 0.12 M Tris base, 0.95 M glycine and 0.5% SDS (w/v) in deionized water. Samples and protein standard were run into well. After finishing the process, gels were then immersed in deionized water for 5 min and stained with coomassie blue stain buffer for 20 minuntes and destained with a destaining buffer for 1 min. The gels were then immersed in 1% acetic acid and incubated for 1 h on a shaker. The acetic acid was replenished every hour until the gel was completely de-stained.

### Statistical Analysis

Only descriptive statistics was utilized for the reporting of the data.

## RESULTS AND DISCUSSION

### Proximate Analysis of Blood Plasma

Table 3 shows the proximate analysis for each blood plasma. The moisture content of catfish blood plasma was 91.46% and 93.05% for the swamp eel.

Table 3
*The proximate analysis of catfish* (Pangasius hypopthalmus) *and swamp eel* (Monopterus albus) *blood plasma*

| Source of plasma | Proximate Analysis | | |
|---|---|---|---|
| | Moisture content (%) | Protein (mg/mL) | Ash (%) |
| Cat Fish | 91.46 | 16.63 | 0.75 |
| Swamp eel | 93.05 | 11.92 | 0.92 |

According to Kisia (2016) and Martini (2005), moisture content in plasma of vertebrates is 90-92%. The difference of moisture content in plasma is due to differences in the chemical composition, such as protein content and ash content, which affects moisture content. Proteins are important components and functions in maintaining water balance in the blood and

tissues, regulating blood volume, helping the transport of fat, vitamins, and hormones and as antibodies.

The protein content of catfish plasma was 16.63 mg/mL and the swamp eel plasma was 11.92 mg/mL. Protein in plasma reflects the blood protein content, except for hemoglobin. Plasma proteins are divided into three main components i albumin (42% w / v), globulin (56%) (α1-globulin, α2-globulin, β-globulin, and γ-globulin) and fibrinogen (1%). The largest proportions contained in solutes include proteins, which account for 6% -8% in blood plasma (Moure et al., 2003). Kisia (2016) suggested that solutes in fish blood plasma varied between freshwater fish and marine fish. The protein content of fish plasma is realtively low when compared to other vertebrates. The blood proteins are important in the maintenance of the right osmotic pressure and viscosity of blood.

The ash content of catfish and swamp eel plasma were 0.75% and 0.92% respectively. The difference of the ash content in plasma is related to endogenous (genetically controlled and associated with species-specific life cycles) and exogenous (such as environmental and dietary) factors (Perschbacher & Stickney, 2013). Ash content reflects the mineral content in blood plasma. Minerals in the blood plasma are part of a minor component of blood plasma which amounts to 1% (Martini, 2005; Moure et al., 2003). Minerals make up the normal extracellular fluid ion composition for vital cellular activity and contributes to the osmotic pressure of body fluids. The major plasma electrolytes are $Na^{+}$, $K^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Cl^{-}$, $HCO^{3-}$, $HPO_4^{2-}$, and $SO_4^{2-}$ (Martini, 2005).

**Inhibitory Activity of Blood Plasma**

The inhibitory activity of blood plasma to protease enzyme increased as the concentration of blood plasma for both fishes increased. The protease inhibitory activity ranged from 7.66 to 50.73% for trypsin and 20.34 to 83.05% for papain for the catfish blood plasma (Figure 1), while the protease inhibitory activity of the swamp eel blood plasma ranged from 9.49 to 46.35% for trypsin and 28.81 to 64.41% for papain (Figure 2).

The blood plasma protease inhibitory activity to papain was higher than that of trypsin. This result was in contrast with the recommendation of Yongswatdigul et al. (2014) and Sriket (2014), where the surimi made from tropical fish were susceptible to serine protease attacks that caused protein degradation. But this was in line with the result of Ge et al., (2014) that reported softening occured in grass carp fillets caused by cathepsin B and L as the major endogenous enzymes leading to proteolytic degradation.

The highest protease inhibitory activity on trypsin and papain was by the swamp eel blood plasma even though its protein concentration was lower than that of the catfish blood plasma (0.15-0.60 mg/mL and 0.21-0.83 mg/mL, respectively). This was probably due to the higher efficacy of the protein plasma of the eel acting as a protease inhibitor as compared to that in

the catfish. Fowler and Park (2015) showed that protease inhibitory activity of salmon plasma ranged between 25% - 81% for papain and below 20% for trypsin. The higher inhibitory activity of the salmon blood plasma as compared to that of the catfish and swamp eel (this study) was probably related to it being free of impurities such as water, fat, ash, and other non-protein components.

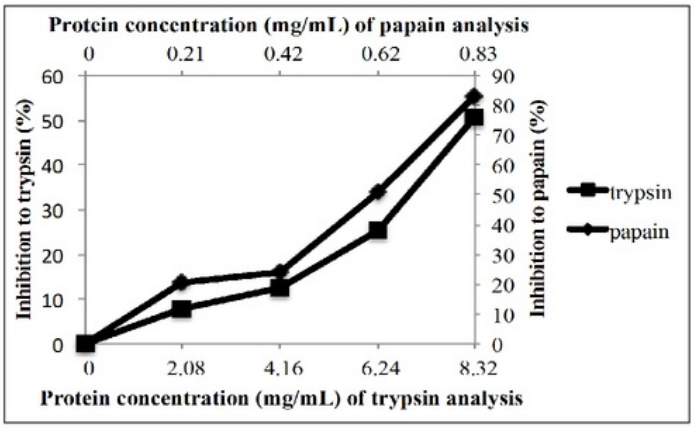


*Figure 1*. Inhibitory activity of catfish (*Pangasius hypopthalmus*) blood plasma to trypsin and papain enzymes

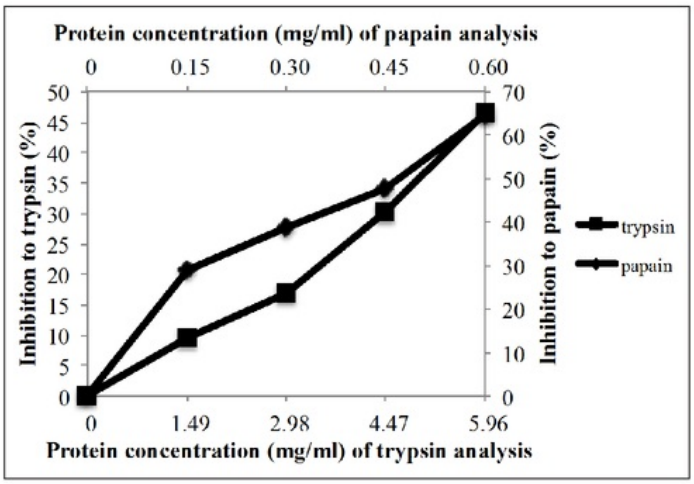


*Figure 2*. Inhibitory activity of swamp eel (*Monopterus albus*) blood plasma to trypsin and papain enzymes

## Molecular Weight of Blood Plasma

The molecular weight of catfish (C1-C3) and swamp eel blood plasma (S1-S3) is in the range of 9.64 to 124.36 kDa (10 proteins) and 19.84 to 174.14 kDa (13 proteins) respectively (Figure 3). The molecular weight of the protein bands detected in the blood plasma of the catfish and swamp eel (this study) showed similarities with

molecular weight of pig plasma. The band is presumed to be α-globulin protein to have molecular weight ranging from 105 kDa - 150 kDa, while albumins had molecular weight of 65 kDa - 69 kDa. $\alpha_2$-globulin is a protease inhibitor found indigenously in blood plasma and shows inhibitory activity againts serine, cysteine, carboxyl and metallo-proteinases (Benjakul et al., 2001; Lee e al., 2000). According to Benjakul and Visessanguan (2000), the serine protease inhibitor is in the range of 58-64 kDa and this is within the range of the proteins in catfish and eel plasma which is able to inhibit the enzymes chymotrypsin and trypsin. Li et al. (2008) reported the presence of the cysteine protease inhibitor at 55 kDa. The variation of the proteins from the catfish and swamp eel determined by the SDS-PAGE analysis reflected their inhibitory activity. The higher variation of the proteins in the swamp eel blood plasma may explain its higher inhibitory activity than that of the catfish.

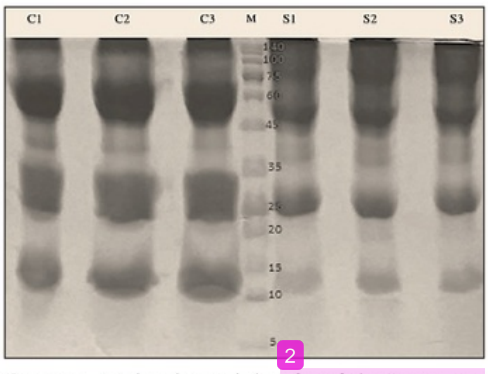


*Figure 3*. Molecular weight of catfish (*Pangasius hypopthalmus*) (C1, C2, C3) and swamp eel (*Monopterus albus*) (S1, S2, S3) blood plasma (M= protein marker)

## CONCLUSION

Both the catfish and swamp eel blood plasma showed inhibitory activity towards trypsin and papain. Papain is a cysteine protease and its inhibition was higher than that of trypsin. The highest inhibitory activity to protease enzyme was from the swamp eel blood plasma.

## ACKNOWLEDGEMENT

The authors wish to thank Sriwijaya University, South Sumatra, Indonesia for the support and the Competitive Research Grant 2017.

## REFERENCES

Association of Official Analytical Chemists. (2005). *Official methods of analyses of the association of analytical chemists* (18th ed.). Washington, USA: Author.

Barret, A. J. (1981). α2-macroglobulin. In S. P. Colowick (Ed.), *Methods in enzymology* (Vol. 80). New York, NY: Academic Press Inc.

Benjakul, S., & Visessanguan, W. (2000). Pig plasma protein: Potential use as proteinase inhibitor for surimi manufacture; inhibitory activity and the active components. *Journal of the Science of Food and Agriculture*, *80*(9), 1351–1356.

Benjakul, S., Srivilai, C., & Visessanguan, W. (2001). Porcine plasma protein as proteinase inhibitor in bigeye snapper (*Priacanthus tayenus*) muscle and surimi. *Journal of the Science of Food and Agriculture*, *81*(10), 1039–1046.

Bradford, M. M. (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. *Analytical Biochemistry*, *72*, 248-254.

Chéret, R., Delbarre-Ladrat, C., de Lamballerie-Anton, M., & Verrez-Bagnis, V. (2007). Calpain

and cathepsin activities in *post mortem* fish and meat muscles. *Food Chemistry*, *101*(4), 1474-1479.

Fowler, M. R., & Park, J. W. (2015). Salmon blood plasma: Effective inhibitor of protease-laden Pacific whiting surimi and salmon mince. *Food Chemistry*, *176*, 448-454.

Ge, L., Xu, Y., & Xia, W. (2014). The function of endogenous cathepsin in quality deterioration of grass carp (*Ctenopharyngodon idella*) fillets stored in chilling condition. *International Journal of Food Science and Technology*, *50*(3), 797-803.

Ho, M., Chen, G., & Jiang, S. (2000). Effect of mackerel cathepsin L and L-like, and calpain on the degradation of mackerel surimi. *Fisheries Science, 66*(3), 558-568.

Jiang, S. T. (2000). Effect of proteinases on the meat texture and seafood quality. *Food Science and Agricultural Chemistry*, *2*(2), 55-74.

Kang, I. S., & Lanier, T. C. (1999). Bovine plasma proteins functions in surimi gelation compared with cysteine protease inhibitors. *Journal of Food Science*, *64*(5), 842–846.

Kisia, S. M. (2016). *Vertebrates structure and functions*. Boca Raton, USA: CRC Press.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680–685.

Lafarga, T., Aluko, R. E., Rai, D. K., O'Cornor, P., & Hayes, M. (2016). Identification of bioactive peptides from a papain hyrolysate of bovine serum albumin and assessment of an antihypertensive effect in spontaneously hypertensive rats. *Food Research International*, *81*, 91-99.

Lafarga, T., Rai, D. K., O'Connor., & Hayes, M. (2015). A bovine fibrinogen-enriched fraction as a source of petides with in vitro renin and angiotensin-i-converting enzyme inhibitory activities. *Journal of Agricultural and Food Chemistry*. *63*(39), 8676-8684.

Lee, J. J., Tzeng, S. S., & Jiang, S. T. (2000). Purification and characterization of low molecular weight kininogen from pig plasma. *Journal of Food Science, 65*(1), 81-86.

Li, D. K., Lin, H., & Kim, S. M. (2008). Purification and characterization of a cysteine protease inhibitor from chum salmon (*Oncorhynchus keta*) plasma. *Journal of Agricultural and Food Chemistry*, *56*(1), 106–111.

Lynch, S. A., Mullen, A. M., O'Neill, E. E., & García, C. Á. (2017). Harnessing the potential of blood proteins as functional ingredients: A review of the state of the art in blood processing. *Comprehensive Reviews in Food Science and Food Safety*, *16*(2), 330-344.

Marquez-Alvarez, L. R., Torres-Arreola, W., Ocano-Higuera, V. M., Ramirez-Wong, B., & Marquez-Rios, E. (2015). Effect of bovine plasma protein on autolysis and gelation pf protein extracted from giant squid (*Dosidicus gigas*) mantle. *Journal of Chemistry*, *2015*, 1-9.

Martini, F. H. (2005). *Anatomy and physiology*. The Metropolis, Singapore: Pearson Education, Inc.

Moure, F., Rendueles, M., & Díaz, M. (2003). Coupling process for plasma protein fraction using ethanol precipitation and ion exchange chromatography. *Meat Science*, *64*(4), 391-398.

Mullen, A. M., Alvarez, C., Pojic, M., Hadnadev, T. D., & Papageorgiou, M. (2015). Classification and target compounds. In C. M. Galanakis (Ed.), *Food waste recovery*. San Diego, USA: Academic Press.

Murray, J., & Burt, J. R. (1983). *The composition of fish*. Aberdeen, Scotland: Torry Research Station.

Nomura, I. (2007). *The state of world fisheries and aquaculture 2006*. Rome, Italy: Food and Agriculture Organization of The United Nations.

Ouali, A. (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie*, *74*(3), 251-265.

Perschbacher, P. W., & Stickney, R. R. (Eds.) (2017). *Tilapia in intensive co-culture*. Chichester, England: John Wiley & Sons Ltd.

Philips, G. O., & Williams, P. A. (2011). *Handbook of food proteins*. Cambridge, United Kingdom: Woodhead Publishing Limited.

Rawdkuen, S., Benjakul, S., Visessanguan, W. & Lanier, T. C. (2007a). Effect of chicken plasma protein and some protein additives on proteolysis and gel-forming ability of sardine (*Sardinella Gibbosa*) surimi. *Journal of Food Processing and Preservation*, *31*(4), 492–516.

Rawdkuen, S., Benjakul, S., Visessanguan, W., & Lanier, T. C. (2007b). Cystein proteinase inhibitor from chicken plasma: Fractination, characterization and autolysis inhibition of fish myofibrillar proteins. *Food Chemistry, 101*(4), 1647-1657.

Sriket, C. (2014). Proteases in fish and shellfish: Role on muscle softening and prevention. *International Food Research Journal, 21*(1), 433-445.

Yongswatdigul, J., Hemung, B. O., & Choi, Y. J. (2014). Proteolytic enzymes and control in surimi. In J. W. Park (Ed.), *Surimi and surimi seafood* (3rd ed.). Boca Raton, USA: Taylor and Francis.

# Protease Inhibitory Activity and Protein Analysis of Catfish (Pangasius hypopthalmus) and Swamp Eel (Monopterus albus) Blood Plasma

ORIGINALITY REPORT

| 31% | 23% | 23% | 16% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Matthew R. Fowler, Jae W. Park. "Salmon blood plasma: Effective inhibitor of protease-laden Pacific whiting surimi and salmon mince", Food Chemistry, 2015<br>Publication | 9% |
| 2 | www.pertanika.upm.edu.my<br>Internet Source | 3% |
| 3 | eprints.unsri.ac.id<br>Internet Source | 3% |
| 4 | R. Nopianti, N. Huda, I. Noryati, A. Fazilah, A.M. Easa. " Cryoprotective effect of low-sweetness additives on protein denaturation of threadfin bream surimi ( spp.) during frozen storage ", CyTA - Journal of Food, 2012<br>Publication | 2% |
| 5 | elibrary.trf.or.th<br>Internet Source | 1% |
| 6 | archimer.ifremer.fr<br>Internet Source | 1% |

| | | |
|---|---|---|
| 7 | www.asianjournalofchemistry.co.in<br>Internet Source | 1% |
| 8 | Submitted to Richmond upon Thames College<br>Student Paper | 1% |
| 9 | www.mdpi.com<br>Internet Source | 1% |
| 10 | Gen-Hung Chen, Shye-Jye Tang, Ching-San Chen, Shann-Tzong Jiang. " High-Level Production of Recombinant Chicken Cystatin by and Its Application in Mackerel Surimi ", Journal of Agricultural and Food Chemistry, 2001<br>Publication | 1% |
| 11 | Wang, Daoying, Muhan Zhang, Shaoying Deng, Weimin Xu, Yuan Liu, Zhiming Geng, Chong Sun, Huan Bian, and Fang Liu. "Postmortem changes in actomyosin dissociation, myofibril fragmentation and endogenous enzyme activities of grass carp (Ctenopharyngodon idellus) muscle", Food Chemistry, 2016.<br>Publication | 1% |
| 12 | psasir.upm.edu.my<br>Internet Source | 1% |
| 13 | Ge, Lihong, Yanshun Xu, Xiaoqing Jiang, Wenshui Xia, and Qixing Jiang. "Broad-spectrum inhibition of proteolytic enzymes by allicin and application in mitigating textural | 1% |

deterioration of ice-stored grass carp (Ctenopharyngodon idella ) fillets", International Journal of Food Science & Technology, 2016.
Publication

| | | |
|---|---|---|
| 14 | onlinelibrary.wiley.com<br>Internet Source | 1% |
| 15 | kb.psu.ac.th<br>Internet Source | 1% |
| 16 | www.scireslit.com<br>Internet Source | <1% |
| 17 | Submitted to Liverpool John Moores University<br>Student Paper | <1% |
| 18 | Submitted to Gyeongsang National University<br>Student Paper | <1% |
| 19 | link.springer.com<br>Internet Source | <1% |
| 20 | Maria Hayes, Michael Gallagher. "Processing and recovery of valuable components from pelagic blood-water waste streams: A review and recommendations", Journal of Cleaner Production, 2019<br>Publication | <1% |
| 21 | P. Khazraiinia, S. Saei, M. Mohri, H. R. Haddadzadeh, H. R. Darvisihha, Z. Khaki. "Serum biochemistry of ostrich (Striothio | <1% |

camelus) in Iran", Comparative Clinical Pathology, 2006
Publication

| | | |
|---|---|---|
| 22 | www.hinter.com.cn<br>Internet Source | <1% |
| 23 | Submitted to University of Luton<br>Student Paper | <1% |
| 24 | Wang, P.A.. "Cathepsin D from Atlantic cod (Gadus morhua L.) liver. Isolation and comparative studies", Comparative Biochemistry and Physiology, Part B, 200707<br>Publication | <1% |
| 25 | jpsr.pharmainfo.in<br>Internet Source | <1% |
| 26 | Soottawat Benjakul. "Porcine plasma protein as proteinase inhibitor in bigeye snapper (Priacanthus tayenus) muscle and surimi", Journal of the Science of Food and Agriculture, 08/2001<br>Publication | <1% |
| 27 | epdf.tips<br>Internet Source | <1% |
| 28 | Submitted to Hadlow College, Kent<br>Student Paper | <1% |
| 29 | baadalsg.inflibnet.ac.in<br>Internet Source | <1% |
| 30 | Submitted to Universiti Teknologi Malaysia | |

| | | |
|---|---|---|
| | Student Paper | <1% |
| 31 | www.freepatentsonline.com<br>Internet Source | <1% |
| 32 | documents.mx<br>Internet Source | <1% |
| 33 | muir.massey.ac.nz<br>Internet Source | <1% |
| 34 | Rawdkuen, S.. "Effect of cysteine proteinase inhibitor containing fraction from chicken plasma on autolysis and gelation of Pacific whiting surimi", Food Hydrocolloids, 200710<br>Publication | <1% |

| | | | |
|---|---|---|---|
| Exclude quotes | Off | Exclude matches | Off |
| Exclude bibliography | On | | |