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Yahoo Mail - Naskah UTM J Teknologi 2015 10 13 an Fronthea

## Naskah UTM J Teknologi 2015 10 13 an Fronthea

Dari: Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc (monika.nur@machung.ac.id)

Kepada: fronthea\_thp@yahoo.co.id

Tanggal: Selasa, 13 Oktober 2015 16.37 WIB

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Demikian yang dapat saya sampaikan. Terima kasih atas perhatiannya.

Salam,

Monika

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**Sent:** Tuesday, October 6, 2015 11:04 AM

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**Subject:** Mohon Komentar Paper

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Salam.

Fronthea Swastawati  
Laboratory of Fisheries Processing Technology  
Faculty of Fisheries and Marine Sciences, Diponegoro University  
Jl. Prof. Sudarto, SH., Tembalang, Semarang 50275  
Phone Office +6224 7474698  
Phone +6281 225 191 989  
+6285 626 816 13

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**CRUDE CATHEPSIN ACTIVITY AND  
QUALITY CHARACTERISTIC OF SMOKED  
CATFISH (*PANGASIU PANGASIU*)  
PROCESSED BY DIFFERENT SMOKING  
TEMPERATURE**

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Fronthea Swastawati<sup>a\*</sup>, Ahmad Ni'matullah Al Baarri<sup>b</sup>,  
Eko Nurcahya Dewi<sup>a</sup>, Ima Wijayanti<sup>a</sup>, Dwi Yanuar Budi  
Prasetyo<sup>c</sup>

\*Corresponding author

fronthea\_thp@undip.ac.id

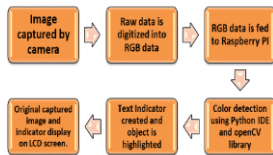
<sup>a</sup>Laboratory of Fish Processing Technology, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Prof Soedarto, SH, Tembalang, Semarang – 50275,  
Indonesia

<sup>b</sup>Laboratory of Chemistry and Food Nutrition, Faculty of  
Animal and Agriculture, Diponegoro University,  
Semarang – 50275, Indonesia

<sup>c</sup>Magister Coastal Resource Management, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Imam Bardjo, SH, Semarang – 50241, Indonesia

**Comment [MNU1]:** Mohon perbaikan kata-kata yang berwarna merah sesuai koreksian pada kotak comment.

## Graphical abstract



## Abstract

Effect of smoking temperature related to the crude cathepsin activity and quality characteristic of smoked catfish (*Pangasius pangasius*) were investigated. Different smoking temperature had significant effect on crude cathepsin activity, texture, pH, moisture content, salt content and solubility of protein. The enzyme had optimum activity at 40-50°C (0.146±0.02 U/mL) but decreasing at 60-70°C (0.114±0.02 U/mL) and 80°C (0.098±0.01 U/mL) respectively. The quality parameters such as textural of smoked catfish were 4591.37±27.12 gforce at 40-50°C; 4241.93±56.82 gforce at 60-70°C; 3881.29±26.75 gforce at 80°C, pH value were 7.42±0.02; 7.72±0.01; 8.01±0.04 respectively, moisture content were 77.42±0.02%; 74.72±0.01%; 72.04±0.04% respectively, salt content were 2.62±0.19%; 3.88±0.33%; 5.83±0.28% respectively and protein solubility were 11.66±0.12%; 8.32±0.30%; 5.80±0.19% respectively. Many factors were correlated to the textural changes of smoked catfish such as changes of crude cathepsin activity, reduction of protein solubility and pH value.

**Keywords :** Crude cathepsin activity, smoking temperature, quality characteristic, catfish (*Pangasius pangasius*)

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### Comment [MNU2]:

Abstrak dilengkapi dengan 'Graphical Abstract' (sesuai contoh di bagian kiri halaman pertama) yang pada dasarnya adalah 'abstrak dalam bentuk gambar'. Graphical abstract dapat berupa diagram alir penelitian ataupun gambar yang representatif.

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0.146 U · mL<sup>-1</sup> ± 0.02 U · mL<sup>-1</sup>

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\*penulisan tanda matematika diberi spasi dari angka di depannya  
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## 1.0 INTRODUCTION

Catfish (*Pangasius pangasius*) is one of the economic aquaculture fish species in Indonesia of which the production was increasing by 37% in 2008-2012 [1]. From the nutrition aspect, catfish contains a high nutrition value to support human health. Catfish contains 35% of protein and 10.1% of lipid with dominant fatty acids profile were lauric acid (13.36%), palmitic acid (26.15%), oleic acid (46.07%) and stearic acid (14.40%) which contributes to human health [2]. Texture is an important quality characteristic and acceptability factor for consumer to consume catfish. The texture of catfish is influenced by several factors such as size, age, macro nutrients composition (protein and lipid content), handling and processing condition. Catfish is one of many aquatic products which is highly perishable, in which after the post mortem the enzymatic decomposition rapidly breaks down the texture of fish components like myofibril and connective tissue. [3], cathepsin enzyme activity could

change the structure and firmness. Cathepsin B, L and D were able to decrease  $\alpha$ -actinin which is responsible to the fish firmness. A significant correlation was observed between enzymatic activity of cathepsin B and L and muscle degradation of Atlantic salmon [4].

1. Smoking process could inhibit the enzymatic decomposition. Brinning, pre drying, chemical composition of smoke and heating in smoking process could change the optimum condition of cathepsin to be active. Cathepsin needs a certain condition to optimize their activity, such as temperature, pH, substrate concentration and the presence of metal inhibitor [5]. Nowadays smoking is not only a preservative method but it also gives a specific flavour and taste on smoked fish to increase the consumer acceptability. This role could be obtained using liquid smoke which is easier to apply and is environmentally safe [6]. The aim of this research was to investigate the effects of smoking temperature to crude cathepsin activity

which affects textural changes and quality characteristic of smoked catfish.

## 2.0 EXPERIMENTAL

### 2.1 Smoking process

Smoking method of catfish was performed with some modification [7]. Fillets of catfish were separated into four groups. Each group dipped into 5% brine and 5% liquid smoke for 30 minutes. Pre-drying in room temperature for about 60 minutes and then smoked in the electrical oven at 40-50°C (P1), 60-70°C (P2) and 80°C (P3) for 1 hour each. Then it was chilled in the room temperature and then packed with polyethylene bag before analysis in the laboratory.

### 2.2 Crude cathepsin activity analysis

Proteolytic activity assay was performed with some modification [8]. One g of fillet was mixed with 1 mL of aquadest and then it was homogenized using centrifuge 600 x g for 10 minutes at 4°C. The supernatant then homogenized again using centrifuge 10.000 x g for 10 minutes at 4°C. Then the extract was dissolved into 1 mL 0.1 M buffer Tris-HCl (Aplichem) pH 7.4, continued with centrifugation at 4.000 x g for 10 minutes at 4°C. Proteolytic activity was analyzed with hemoglobin 2% pH 2 (Oxoid) for the substrate. Substrate solution (0.5 mL) was incubated with 0.1 mL enzyme solution in 37°C for 10 minutes. 2 mL TCA 5% was added and then filtered. The solution gained was filtrated, and then 1 mL Folin reaction was added. Final solution was read in spectrophotometry on 750nm, blank and standard solutions (Tirosin) were read in the same wavelength.

### 2.3 Quality characteristic analysis

#### 2.3.1 Texture analysis

The fillet texture were measured using Texture analyzer TA – TX2. The probe was pressed into the fillet at a constant speed of 2 mm/s until it reached 60% of the sample height. The maximum force obtained during compression (gforce) was recorded.

#### 2.3.2 pH, moisture content and salt content

The 10 g of samples were homogenized with 90mL aquadest for 1 min. The electrode of pH meter (pH meter Hanna Instrument) was inserted into the slurry while being stirred vigorously. After stabilization, the observed value was recorded. Moisture content of samples were measured at 105°C according to the gravimetric test. The salt content was determined using Silver Nitrite Method.

#### 2.3.3 Solubility protein analysis

Protein content in supernatant was divided into two groups of experiment, i.e. set I and set II. Set I was performed with Biuret reaction and set II was using alkaline cooper sulphate reagent. Color was measured using spectrophotometer at 540 nm. Bovine serum albumin was used as the standard solution. All the analysis were run in duplicate.

### 2.4 Statistical Analysis

Group Randomly Design was used in this research and analysis was performed using ANOVA with significant level of 95% and 99%. The computer software for helping this project is SPSS ver 20.

## 3 RESULTS AND DISCUSSION

### 3.1 Crude cathepsin activity

Crude cathepsin activity on raw catfish was 0,144 and it is comparable with previous experiment [5], which showed that crude chatepsin activity on catfish after post rigor was 0.278 U/mL. Different results indicated that activity of crude cathepsin in the same species was not the same due to several factors such as sexual maturity level. The highest cathepsin value is at sexual maturity level [9]. The statistical analysis showed that smoking temperature gave significant different for crude cathepsin activity. Based on Tukey and LSD test each smoking temperature did not gave significant different than the other. The results (0,146 – 0,098 U/mL) showed that different smoking temperature reduce crude

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chatepshin activity on smoked catfish fillets (Table 1).

2. Crude cathepsin activity was decreasing with the increasing of smoking temperature. The highest value of crude cathepsin activity was on smoked catfish at P1 (40-50°C) then decreasing at 60-70°C and 80°C for P2 and P3 respectively. The reducing activity of crude cathepsin was caused by combination of salt, heat and smoke in catfish smoking process by changing the optimum condition of enzyme to be active (See Table 2). Similar with previous experiment [5], the optimum condition to optimize activity of crude cathepsin are temperature, pH and metals inhibitor. The optimum temperatures were 40-50°C and decreasing rapidly with increasing temperature in 60-70°C. Optimum pH for crude cathepsin activity are 6-7, the metals inhibitor like Na have 85% relative activity to inhibit the cathepsin activity. Gross Proteolytic Activity of smoked salmon which smoked at 20-30°C was 0.535 mg peptides/mg [10].

Table 1. Crude cathepsin activity (U/mL)

Treatment	Value
P0 (Control)	0,144±0,01 <sup>a</sup>
P1	0,146±0,02 <sup>ab</sup>

Table 2. Texture (gforce), pH, moisture content (wet basis %), salt content (wet basis %) and protein solubility (%) of smoked fillets catfish.

beri keterangan di kolom ini	P0	P1	P2	P3
Texture	5482.43±93.45 <sup>a</sup>	4591.37±27.12 <sup>b</sup>	4241.93±56.82 <sup>c</sup>	3881.29±26.75 <sup>d</sup>
pH	7.22±0.04 <sup>a</sup>	7.42±0.02 <sup>b</sup>	7.72±0.01 <sup>c</sup>	8.01±0.04 <sup>d</sup>
Moisture content	79.66±0.04 <sup>a</sup>	77.42±0.02 <sup>b</sup>	74.72±0.01 <sup>c</sup>	72.04±0.04 <sup>d</sup>
Salt content	1.43±0.10 <sup>a</sup>	2.62±0.19 <sup>b</sup>	3.88±0.33 <sup>c</sup>	5.83±0.28 <sup>d</sup>
Protein Solubility	14.37±0.42 <sup>a</sup>	11.66±0.12 <sup>b</sup>	8.32±0.30 <sup>c</sup>	5.80±0.19 <sup>d</sup>

Note : Value from average of duplicate ± deviation standar

Value following with same small superscript letters were no significantly different (p>0.05).

P2	0,114±0,02 <sup>abc</sup>
P3	0,098±0,01 <sup>abc</sup>

\*Note: Value from average of duplicate ± deviation standar

\*\*Value following with same small superscript letters were no significantly different (p>0.05)

### 3.2 Texture

Texture analysis were shown in Table 2, based on Analysis of Varian, different smoking temperature gave significant effect (p<0.05) to the texture of smoked catfish.

In this study the textural changes of smoked catfish caused by smoking temperature and protein solubility (see solubility protein analysis) were collected. The textural value (Table 2) were decreasing while the smoking temperature was increasing. Heat treatments lead to denaturation on protein muscle, long heat treatment will form aggregation [11]. The textural change was caused by denaturation of protein muscle, then the water soluble protein became semisolid gel structure and it hardened the fish texture.

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With increasing temperature, textural value decreased (see Table 2).

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The textural changes of smoked catfish were caused by crude cathepsin activity, although little effect, it all was caused by cathepsin activity. Many proteins which correlates to firmness were affected by cathepsins and make textural changes on rainbow trout [12]. This enzyme was responsible for tissue degradation. The range of fibre densities (85 – 140 fibre mm<sup>-2</sup>) were observed more "chewiness" and "firmness" in texture charteristics of smoked salmon. The high fibre densities contribute to a firmer texture in fish muscle, and the variety of fibre densities in fish is affected by sexual maturity.

3.3 pH

The pH values of smoked catfish were shown in Table 2. The fresh catfish had pH values of 7.22±0.04, and increased after smoking process. pH values of smoked fish from this study were 7.42-8.01. According to previous research on smoked stingray, pH values of smoked stingray using cocconut shell and corn cob liquid smoke respectively were 7.30 and 8.20 [6]. pH changes on smoked fish is affected by chemical composition of liquid smoke [13]. pH is an important parameter to determine enzyme activity in which pH affects the ionization condition which need the bounding between substrate and enzyme. The catalysis reaction depends on interaction between substrate with side chains amino acid which compile the active side of enzyme [14]. The cathepsin were optimum at pH 6 with the cathepsin activity value of 0.271 U/mL, but in pH 7 or above the value of the cathepsin activity decreased with the value of 0.167 U/mL [5].

3.4 Moisture content

The results of moisture content of smoked catfish were 72.04 – 77.33%, while the moisture content of raw fish was 79.66%. Smoking process could reduce the moisture content of smoked fish due to combination of salting, pre drying and heating process which could evaporate the moisture in fish muscle. Previous research showed that moisture content of smoked milkfish processed by corn cob liquid smoke and then heated in electrical oven and mechanical oven for 3 hours were 58.33% and 63.37% [7].

3.5 Salt content

The salt content of smoked catfish was 2.62 – 5.83%. There was a slight increase in the value of salt content with the increasing of smoking temperature (p<0.05). While smoking temperature increased, moisture evaporated and then the salt penetrated into fish flesh because of the osmosis effect from salt. In other experiment, salt content of smoked salmon were 4.0 – 7.2 g per 100 g mositure. While salt content of smoked sea bass which was processed by spray with liquid smoke for 30 minutes and added with NaCl (0.110; 0.150; 0.200; 0.220; 0.270 (g solution of NaCl / g dry material) showed that the salt content were 2.05; 4.46; 4.83; 9.40 dan 17.76 [10, 15].

3.6 Solubility Protein

The solubility protein of smoked catfish decreased as a result of increasing smoking temperature, the heating process indicated the changes on protein solubility. The denaturation of protein was related to protein solubility, the tersier or secondary structure of protein damaged into primary structure by heat treatments. In the primary structure both of the water or salt soluble protein were released easier than in tersier or secondary structure. The decreasing solubility of protein happens as well as by the increasing of smoking temperature. [10], the protein solubility value of smoked salmon which smoked at 29.9°C were significantly lower than smoked salmon smoked at 21.5°C. Another study showed that by applying different process on mullets fillet with high temperature until lower temperature heating i.e. frying (150°C for 10 mins), grilling (100°C for 20 min, 10 min on each side) and steaming (98°C for 20 min), showed that the highest solubility of protein at frying process, grilling and steaming respectively [16].

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## 4 CONCLUSION

Increasing smoking temperature could inhibit the crude cathepsin enzyme activity, reduced the texture, moisture content, and protein solubility of smoked catfish. The information from this study could be a reference for producing the quality smoked fish in particular on characteristic of protein nutritive aspect.

## Acknowledgements

The authors would like to acknowledge the financial support provided by Directorate General of Research and Public Services, Ministry of Research, Technology and Higher Education with contract number DIPA-023.04.1.673453/2015, November 14<sup>th</sup> 2014 revised on DIPA March 1<sup>st</sup> 2015.

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Penulisan referensi dari jurnal yang benar:

Islam, R., Paul, D.K., Rahman, A., Parvin, T., Islam, D., Sattar, A. 2012. Comparative Characteristic of Lipids and Nutrient Contents of *Pangasius pangasius* and *Pangasius sutchi* Available in Bangladesh. *Journal Nutrition & Food Science*. 2(2): 130–135.

\*pemisah antara nama author, tahun, judul paper, nama jurnal dan volume menggunakan tanda titik

\*nama author  
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\*tahun  
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- huruf pertama pada setiap kata menggunakan huruf capital, kecuali pada kata depan (eg; in, on, of, and, dll ditulis huruf kecil)  
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10/20/2020

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## Re: Koreksian naskah a.n. Frontha Swastawati

Dari: Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc (monika.nur@machung.ac.id)

Kepada: frontha\_thp@yahoo.co.id

Tanggal: Jumat, 6 November 2015 14.30 WIB

yth. Bu Thea,

Saya melampirkan koreksian kembali. Tinggal sedikit koreksiannya, Bu.

Untuk komentar dari MB materi (Bu Kapti Rahayu) pada dasarnya merekomendasikan untuk dipublish. Komentar beliau hanya seputar format penulisan satuan dan referensi sementara dari materi sudah dinilai baik.

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1. Untuk satuan gforce, kalau memang ada padanan Slnya mungkin sebaiknya diganti Bu. Tapi saya cari juga sepertinya susah ya Bu. Mungkin kasus ini masih tidak apa-apa kalau tidak diganti Bu.
2. Maaf Bu Thea, untuk pH memang tidak ada satuannya. Sepertinya saya salah memberi coretan di naskah. Jadi untuk yang pH tidak ada masalah Bu.

Demikian dari saya. Mohon maaf agak lama merespon email Ibu. Saya tunggu revisinya kembali.

Terima kasih

Monika

**From:** Frontha Swastawati <frontha\_thp@yahoo.co.id>

**Sent:** Monday, November 2, 2015 3:16 PM

**To:** Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc

**Subject:** Bls: Koreksian naskah a.n. Frontha Swastawati

Ybs. Mbak Monika

Bersama ini kami lampirkan paper yang sudah kami perbaiki berdasarkan koreksi dari mbak monika. Namun ada beberapa pertanyaan / mohon masukan dari Mbak Monika seperti :

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salam,  
Frontha Swastawati

10/20/2020

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Pada Kamis, 29 Oktober 2015 14:32, "Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc" <monika.nur@machung.ac.id> menulis:

yth. Ibu Fronthea,

Saya melampirkan naskah Ibu kembali yang telah saya koreksi lagi format penulisannya. Mungkin sekalian dengan templatnya supaya bisa dijadikan acuan ya Bu. Mohon berkenan menyelesaikan revisian dan mengirimkan kembali kepada saya selambatnya tanggal 3 November 2015.

Berkenaan dengan data baru yang Ibu tambahkan di naskah, maka saya kembali mengirimkan naskah Ibu kepada Ibu Kapti Rahayu untuk diperiksa sekali lagi.

Demikian yang dapat saya sampaikan. Terima kasih atas perhatiannya.

Salam,

Monika

---

**From:** Fronthea Swastawati <fronthea\_thp@yahoo.co.id>

**Sent:** Thursday, October 22, 2015 12:25 PM

**To:** Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc

**Subject:** Jurnal\_Enzyme\_Dr.Fronthea\_221015

Yth. Mbak Monika

Selamat siang mbak monika, bersama ini saya kirimkan jurnal enzyme yang sudah saya perbaiki dan ada tambahan data mengenai SEM. mohon sekiranya bisa dikoreksi kembali. Atas perhatian dan kerjasamanya yang baik saya ucapkan terima kasih.

Salam.

Fronthea Swastawati  
Laboratory of Fisheries Processing Technology  
Faculty of Fisheries and Marine Sciences, Diponegoro University  
Jl. Prof. Sudarto, SH., Tembalang, Semarang 50275  
Phone Office +6224 7474698  
Phone +6281 225 191 989  
+6285 626 816 13

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**CRUDE CATHEPSIN ACTIVITY AND  
QUALITY CHARACTERISTIC OF SMOKED  
CATFISH (*PANGASIU PANGASIU*)  
PROCESSED BY DIFFERENT SMOKING  
TEMPERATURE**

## Article history

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Fronthea Swastawati<sup>a\*</sup>, Ahmad Ni'matullah Al Baarri<sup>b</sup>,  
Tri Winarni Agustini<sup>a</sup>, Eko Nurcahya Dewi<sup>a</sup>, Ima  
Wijayanti<sup>a</sup>, Dwi Yanuar Budi Prasetyo<sup>c</sup>

\*Corresponding author

fronthea\_thp@undip.ac.id

<sup>a</sup>Laboratory of Fish Processing Technology, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Prof Soedarto, SH, Tembalang, Semarang – 50275,  
Indonesia

<sup>b</sup>Laboratory of Chemistry and Food Nutrition, Faculty of  
Animal and Agriculture, Diponegoro University,  
Semarang – 50275, Indonesia

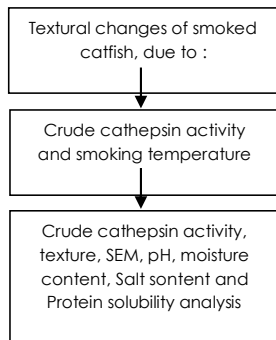
<sup>c</sup>Magister Coastal Resource Management, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Imam Bardjo, SH, Semarang – 50241, Indonesia

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## Graphical abstract



## Abstract

The aim of this research were to investigate the effect of smoking temperature towards crude cathepsin activity and quality characteristic of smoked catfish (*Pangasius pangasius*). Different smoking temperature had significant effect ( $p < 0.05$ ) on crude cathepsin activity, texture, pH, moisture content, salt content and protein solubility. The significant decreasing (30.13 %) of crude cathepsin activity at P3 (80 °C) from P1 (40 °C to 50 °C). Many factors were correlated to the textural changes of smoked catfish such as changes of crude cathepsin activity, reduction of protein solubility and pH value.

**Keywords :** Catfish (*Pangasius pangasius*), crude cathepsin activity, quality characteristic, smoking temperature

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## 3.0 INTRODUCTION

Catfish (*Pangasius pangasius*) is one of the economic aquaculture fish species that can be found in Indonesia's waters. Its production was increasing by 37 % during 2008 to 2012 [1]. In terms of its nutrition aspect, catfish contains about 35 % of protein and 10.1 % of lipid with dominant fatty acids profile were lauric acid (13.36 %), palmitic acid (26.15 %), oleic acid (46.07 %) and stearic acid (14.40 %) which contribute to human health [2]. Texture is an important quality characteristic and acceptability factor for consumer. The texture of catfish is influenced by several factors such as size, age, macro nutrients composition (protein and lipid content), handling and processing condition. Catfish is one of many aquatic products which is highly perishable, in which after the post mortem the enzymatic decomposition rapidly breaks down the texture of fish components like myofibril and connective tissue. [3], cathepsin enzyme activity could change the structure and firmness. Cathepsin B, L and D were able to decrease  $\alpha$ -actinin which is responsible to the fish firmness. A significant correlation was observed between enzymatic activity of cathepsin B and L and muscle degradation of Atlantic salmon [4].

Smoking process could inhibit the enzymatic decomposition. Brinning, pre drying, chemical composition of smoke and heating in smoking

process could change the optimum condition of cathepsin to be active. Cathepsin needs a certain condition to optimize their activity, such as temperature, pH, substrate concentration and the presence of metal inhibitor [5]. Nowadays smoking is not only a preservative method but it also gives a specific flavour and taste on smoked fish to increase the consumer acceptability. This role could be obtained using liquid smoke, which is easier to apply and is environmentally safe [6]. The aim of this research was to investigate the effects of smoking temperature to crude cathepsin activity which affect textural changes and quality characteristic of smoked catfish.

## 4.0 EXPERIMENTAL

### 2.1 Smoking Process

Smoking method of catfish was performed with some modification [7]. Fillets of catfish were separated into four groups. Each group dipped into 5 % brine and 5 % liquid smoke for 30 min. Pre-drying in room temperature for about 60 min and then smoked in the electrical oven at 40 °C to 50 °C (P1), 60 °C to 70 °C (P2) and 80 °C (P3) for 1 h each. Then it was chilled in the room temperature and then packed with polyethylene bag before analysis in the laboratory.

### 3.2 Crude Cathepsin Activity Analysis

Proteolytic activity assays was performed with some modification [8]. One g of fillet was mixed with 1 mL of aquadest and then it was separated using centrifugation at  $600 \times g$  for 10 min at  $4^\circ\text{C}$ . The supernatant was then separated again using centrifugation at  $10,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Then the extract was dissolved into 1 mL 0.1 M Tris-HCl buffer (Aplichem) pH 7.4, continued with centrifugation at  $4,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Proteolytic activity was analyzed with hemoglobin 2 % pH 2 (Oxoid) for the substrate. Substrate solution (0.5 mL) was incubated with 0.1 mL enzyme solution at  $37^\circ\text{C}$  for 10 min. Two mL Trichloro Acetic Acid (TCA) 5% was added and then filtered. The solution gained was filtrated, and then 1 mL Folin reaction was added. Final solution was read in spectrophotometry on 750 nm, blank and standard solutions (Tyrosin) were read in the same wave length.

### 3.3 Quality Characteristic Analysis

#### 3.3.1 Texture Analysis

Texture were measured using Texture analyzer TA – TX2. The probe was pressed into the fillet at a constant speed of  $2 \text{ mm} \cdot \text{s}^{-1}$  until it reached 60 % of the sample height. The maximum force obtained during compression (gf) was recorded.

#### 3.3.2 pH, Moisture Content and Salt Content

Ten g of samples were homogenized with 90 mL aquadest for 1 min. The electrode of pH meter (pH meter Hanna Instrument) was inserted into the slurry while being stirred vigorously. After stabilization, the observed value was recorded. Moisture content of samples were measured at  $105^\circ\text{C}$  according to the gravimetric test. The salt content was determined using Silver Nitrite Method.

#### 3.3.3 Protein Solubility Analysis

Protein content in supernatant was divided into two groups of experiment, i.e. set I and set II. Set I

was performed with Biuret reaction and set II was using alkaline cooper sulphate reagent. Color was measured using spectrophotometer at 540 nm. Bovine serum albumin was used as the standard solution. All the analysis were run in duplicate.

### 3.4 Statistical Analysis

Group Randomly Design was used in this research and analyzed using ANOVA with significant level of 95 %. The computer software for helping this project was SPSS ver 20.

## 5.0 RESULTS AND DISCUSSION

### 5.1 Crude Cathepsin Activity

Crude cathepsin activity on raw catfish was  $0.860 \text{ U} \cdot \text{mL}^{-1}$  and it is comparable with previous experiment [5], which showed that crude cathepsin activity on cat fish after post rigor was  $0.278 \text{ U} \cdot \text{m}^{-1}$ . The various results might be influenced by several factors such as sexual maturity level. The highest cathepsin value was reached at sexual maturity season [9]. The statistical analysis showed that smoking temperature gave significant differences for crude cathepsin activity. Based on LSD test the smoking temperature give significant different for crude cathepsin activity. The results showed that P0 and P1 did not significant different, P1 significant different with P2 and P3 but P2 did not significant different with P3 (Table 1). Reduction of crude cathepsin activity showed significant at P3 (31.98 %) from P0; 21.16 % from P0 to P2 and 13.72 % from P2 to P3.

**Table 1** Crude cathepsin activity ( $\text{U} \cdot \text{mL}^{-1}$ )

Treatment	Value
P0 (Control)	$0.860 \pm 0.06^a$
P1	$0.880 \pm 0.03^a$
P2	$0.678 \pm 0.00^b$

P3	0.585 ± 0.02 <sup>b</sup>
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Note: Average value of duplicate ± standard deviation

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

Crude cathepsin activity was decreasing with the increasing of smoking temperature. The highest value of crude cathepsin activity was on smoked catfish at P1 (40 °C to 50 °C) then followed by P2 (60 °C to 70 °C) and P3 (80 °C). The reduction of crude cathepsin activity may be affected by combination of salt, heat and smoke in catfish smoking process by changing the optimum condition of enzyme to be active (Table 2). It was similar with previous experiment [5] that, the optimum condition to optimize activity of crude cathepsin were temperature, pH and metals inhibitor. The optimum temperatures were 40 °C to 50 °C and

decreasing rapidly with increasing temperature in 60 °C to 70 °C. The value of optimum pH for crude cathepsin activity was recorded in the range 6 to 7, the metals such as Na could inhibit 85 % relative cathepsin activity. Gross Proteolytic Activity of smoked salmon which smoked at 20 °C to 30 °C was 0.535 mg peptides  $\text{mg}^{-1}$  [10].

### 3.2 Texture

Based on the data that was shown at Table 2 smoking temperature and protein solubility caused the changes on smoked catfish texture. The increase of smoking temperature affected the decrease in the textural value. Heat treatments lead to denaturation on protein muscle, long heat treatment will form aggregation [11]. The textural change caused by denaturation of protein muscle, then the water soluble protein and the texture tended to semisolid gel structure resulting in the hard texture.

**Table 2** Texture (gfi), pH, moisture content (wet basis %), salt content (wet basis %) and protein solubility (%) of smoked fillets catfish

Parameters	P0	P1	P2	P3
Texture	5482.43 ± 93.45 <sup>a</sup>	4591.37 ± 27.12 <sup>b</sup>	4241.93 ± 56.82 <sup>c</sup>	3881.29 ± 26.75 <sup>d</sup>
pH	7.22 ± 0.04 <sup>a</sup>	7.42 ± 0.02 <sup>b</sup>	7.72 ± 0.01 <sup>c</sup>	8.01 ± 0.04 <sup>d</sup>
Moisture content	79.66 ± 0.04 <sup>a</sup>	77.42 ± 0.02 <sup>b</sup>	74.72 ± 0.01 <sup>c</sup>	72.04 ± 0.04 <sup>d</sup>
Salt content	1.43 ± 0.10 <sup>a</sup>	2.62 ± 0.19 <sup>b</sup>	3.88 ± 0.33 <sup>c</sup>	5.83 ± 0.28 <sup>d</sup>
Protein Solubility	14.37 ± 0.42 <sup>a</sup>	11.66 ± 0.12 <sup>b</sup>	8.32 ± 0.30 <sup>c</sup>	5.80 ± 0.19 <sup>d</sup>

Note : Average value of of duplicate measurement ± standard deviation.

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

The textural changes of smoked catfish were caused by crude cathepsin activity, even in small amount. Cathepsin activity was correlated to firmness and made textural changes on rainbow trout [12]. This enzyme was responsible for tissue degradation. The range of fibre densities from 85 fibre  $\text{mm}^{-2}$  to 140 fibre  $\text{mm}^{-2}$  indicates

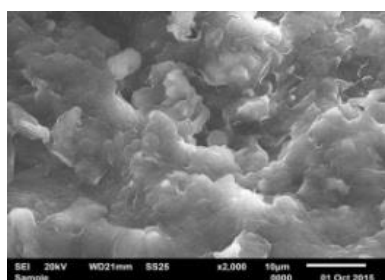
optimum "chewiness" and "firmness" in texture characteristics of smoked salmon. The high fibre densities contributed to a firmer texture in fish muscle, and the variety of fibre densities in fish was affected by sexual maturity [13].

### 3.3 Scanning Electron Microscope (SEM)

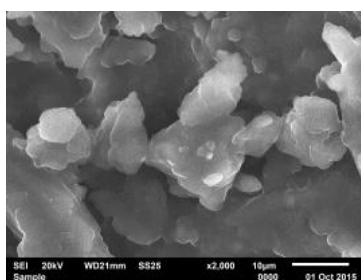
Textural changes of smoked catfish were measured by Scanning Electron Microscope (SEM). SEM analysis was performed to describe structural changes in texture of smoked catfish. Figure 1(a) showed that the structure of texture smoked catfish still complex and solid. Figure 1 (b) showed that the texture molecules of smoked catfish more visible aggregate. Figure 1 (c) the clods more bigger concomitant with increasing time and smoking temperature. At the end of smoking process (Figure 1d) the clods of texture smoked catfish were destroyed and the structure

became loose. In the previous research, the aggregate formation increased regularly in meat heated at 60 °C, whereas meat heated at higher temperature (100 °C and 140 °C) showed dramatic increase up to 5-fold the initial level. The temperature increase promote exposure the thiol group and interior hydrophobic residues of BSA, enabling the formation of hydrogen bonds and hydrophobic interactions. This reaction and interactions promoted protein aggregation via a non-native and expanded conformational state [11, 14].

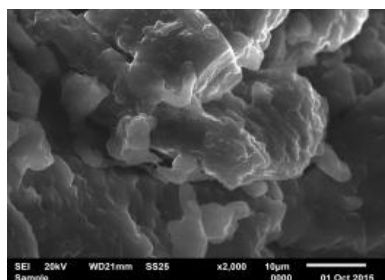
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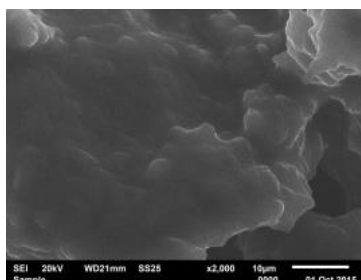
1 (a)



1 (b)



1 (c)



1 (d)

Figure 1. SEM image from smoked catfish with various treatments, i.e. (a) P0, (b) P1, (c) P2 and (d) P3

### 3.4 pH

The pH values of smoked catfish were shown in Table 2. The fresh catfish had pH values of  $7.22 \pm 0.04$ , and increased after smoking process. pH values of smoked fish from this study were 7.42 to 8.01. According to previous research on smoked stingray, pH values of smoked stingray using cocconut shell and corn cob liquid smoke were 7.30 and 8.20 respectively [6]. The changes on pH on smoked fish was affected by chemical composition of liquid smoke [15]. The value of pH is an important role to determine enzyme activity in which pH affects the ionization condition which needs the bounding between substrate and enzyme. The catalysis reaction depended on interaction between substrate with side chains amino acid which bound the active side of enzyme [16]. When pH of cathepsin was 6, cathepsin activity was  $0.271 \text{ U} \cdot \text{mL}^{-1}$ , but in pH 7 or above the value of the cathepsin activity decreased ( $0.167 \text{ U} \cdot \text{mL}^{-1}$ ) [5]. This irreversible enzyme was inactive at pH above 7 but generally highly active at acidic environment [17].

### 3.5 Moisture Content

Moisture content of smoked catfish were 72.04 % to 77.33 %, while the moisture content of raw fish was 79.66 %. Smoking process caused the reduction in the moisture content of smoked fish and, the combination of salting, pre drying and heating process evaporated the moisture in fish muscle as well. Previous research showed that moisture content of smoked milkfish processed by corn cob liquid smoke was 58.33 % and 63.37 % respectively [7].

### 3.6 Salt Content

The salt content of smoked catfish was 2.62 % to 5.83 %. There was a slight increase in the value of salt content with the increasing of smoking temperature ( $p < 0.05$ ). While smoking temperature increased, moisture evaporated and then the salt penetrated into fish flesh because of the osmosis effect from salt. In other experiment, salt content of smoked salmon were 4.0 g to 7.2 g per 100 g moisture [10]. While salt content of smoked sea bass which were processed by spray with liquid smoke for 30 min and added with NaCl (0.110 %; 0.150 %; 0.200 %; 0.220 %; 0.270 %) were 2.05 %; 4.46 %; 4.83 %; 9.40 % and 17.76 % respectively [18].

### 3.7 Protein Solubility

Protein solubility of smoked catfish decreased as a result of increasing smoking temperature, the heating process indicated the changes on protein solubility. The denaturation of protein relates to protein solubility, the tertiary or secondary structure of protein was damaged until the became primary structure due to heat treatments. In the primary structure, both water and salt soluble protein were released easier than in tertiary or secondary structure. The decreasing solubility of protein occurred because of increased smoking temperature. In the previous research, the protein solubility value of smoked salmon which was smoked at  $29.9^\circ\text{C}$  were significantly lower than salmon smoked at  $21.5^\circ\text{C}$  [10]. The changes of protein solubility was due to pH in which protein solubility increased at extremely acidic and alkaline environment. Previous research showed that at the extreme of pH, solubility increased to almost five times that of the original (pH 6.3), i.e.  $125.73 \pm 0.64 \text{ mg} \cdot \text{g}^{-1}$  at pH 2;  $58.92 \pm 1.10 \text{ mg} \cdot \text{g}^{-1}$  at pH 4;  $21.42 \pm 0.5 \text{ mg} \cdot \text{g}^{-1}$  at pH 6;  $44.76 \pm 0.95 \text{ mg} \cdot \text{g}^{-1}$  at pH 8 and  $122.85 \pm 1.2 \text{ mg} \cdot \text{g}^{-1}$  at pH 12 [19]. Another study showed that minimum protein solubility that called isoelectric point, in raw or cooked samples exhibited at pH 5 to 6. Protein solubility decreased with increasing pH to isoelectric point then increased again to high pH [20].



## 4.0 CONCLUSION

Inceasing smoking temperature could inhibit the crude cathepsin enzyme activity and reduced the texture, moisture content, and protein solubility of smoked catfish. The information from this study could be a reference to produce good quality smoked fish in particular protein nutritive aspect.

## Acknowledgements

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Contoh:

Fikri, MZ., Nurhayati, T. and Salamah, E. 2014. Judul dalam Bahasa Indonesia [Extraction and Partial Characterization of Crude Cathepsin from Catfish]. *J. Teknol. dan Industri Pangan*. 25 (1) : 119–123 [Bahasa Indonesia]

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10/20/2020

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## Naskah a.n. Fronthe (2015.12.03)

Dari: Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc (monika.nur@machung.ac.id)

Kepada: fronthea\_thp@yahoo.co.id

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Salam,

Monika

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**From:** Fronthea Swastawati <fronthea\_thp@yahoo.co.id>

**Sent:** Wednesday, December 2, 2015 1:23 PM

**To:** Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc

**Subject:** Enzyme\_Dr.Fronthea\_02122015

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Salam.

Fronthea Swastawati  
Laboratory of Fisheries Processing Technology  
Faculty of Fisheries and Marine Sciences, Diponegoro University  
Jl. Prof. Sudarto, SH., Tembalang, Semarang 50275  
Phone Office +6224 7474698  
Phone +6281 225 191 989  
+6285 626 816 13

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# CRUDE CATHEPSIN ACTIVITY AND QUALITY CHARACTERISTIC OF SMOKED CATFISH (*PANGASIU PANGASIU*) PROCESSED BY DIFFERENT SMOKING TEMPERATURE

## Article history

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Fronthea Swastawati<sup>a\*</sup>, Ahmad Ni'matullah Al Baarri<sup>b</sup>, Tri  
Winarni Agustini<sup>a</sup>, Eko Nurcahya Dewi<sup>a</sup>, Ima Wijayanti<sup>a</sup>,  
Dwi Yanuar Budi Prasetyo<sup>c</sup>

<sup>a</sup>Laboratory of Fish Processing Technology, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Prof Soedarto, SH, Tembalang, Semarang – 50275,  
Indonesia

<sup>b</sup>Laboratory of Chemistry and Food Nutrition, Faculty of  
Animal and Agriculture, Diponegoro University, Semarang  
– 50275, Indonesia

<sup>c</sup>Magister Coastal Resource Management, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Imam Bardjo, SH, Semarang – 50241, Indonesia

\*Corresponding author

fronthea\_thp@undip.ac.id

Tel. +6281225191989

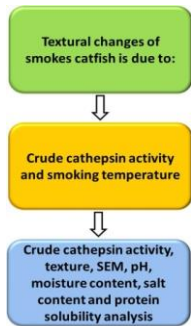
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## Graphical abstract



## Abstract

The aim of this research were to investigate the effect of smoking temperature towards crude cathepsin activity and quality characteristic of smoked catfish (*Pangasius pangasius*). Different smoking temperature had significant effect ( $p < 0.05$ ) on crude cathepsin activity, texture, pH, moisture content, salt content and protein solubility. The significant decreasing (30.13 %) of crude cathepsin activity at P3 (80 °C) from P1 (40 °C to 50 °C). Many factors were correlated to the textural changes of smoked catfish such as changes of crude cathepsin activity, reduction of protein solubility and pH value.

**Keywords :** Catfish (*Pangasius pangasius*), crude cathepsin activity, quality characteristic, smoking temperature

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## 6.0 INTRODUCTION

Catfish (*Pangasius pangasius*) is one of the economic aquaculture fish species that can be found in Indonesia's waters. Its production was increasing by 37 % during 2008 to 2012 [1]. In terms of its nutrition aspect, catfish contains about 35 % of protein and 10.1 % of lipid with dominant fatty acids profile were lauric acid (13.36 %), palmitic acid (26.15 %), oleic acid (46.07 %) and stearic acid (14.40 %) which contribute to human health [2]. Texture is an important quality characteristic and acceptability factor for consumer. The texture of catfish is influenced by several factors such as size, age, macro nutrients composition (protein and lipid content), handling and processing condition. Catfish is one of many aquatic products which is highly perishable, in which after the post mortem the enzymatic decomposition rapidly breaks down the texture of fish components like myofibril and connective tissue. [3], cathepsin enzyme activity could change the structure and firmness. Cathepsin B, L and D were able to decrease  $\alpha$ -actinin which is responsible to the fish firmness. A significant correlation was observed between enzymatic activity of cathepsin B and L and muscle degradation of Atlantic salmon [4].

Smoking process could inhibit the enzymatic decomposition. Brining, pre drying, chemical composition of smoke and heating in smoking process could change the optimum condition of cathepsin to be active. Cathepsin needs a certain

condition to optimize their activity, such as temperature, pH, substrate concentration and the presence of metal inhibitor [5]. Nowadays smoking is not only a preservative method but it also gives a specific flavour and taste on smoked fish to increase the consumer acceptability. This role could be obtained using liquid smoke, which is easier to apply and is environmentally safe [6]. The aim of this research was to investigate the effects of smoking temperature to crude cathepsin activity which affect textural changes and quality characteristic of smoked catfish.

## 7.0 EXPERIMENTAL

### 2.1 Smoking Process

Smoking method of catfish was performed with some modification [7]. Fillets of catfish were separated into four groups. Each group dipped into 5 % brine and 5 % liquid smoke for 30 min. Pre-drying in room temperature for about 60 min and then smoked in the electrical oven at 40 °C to 50 °C (P1), 60 °C to 70 °C (P2) and 80 °C (P3) for 1 h each. Then it was chilled in the room temperature and then packed with polyethylene bag before analysis in the laboratory.

### 3.5 Crude Cathepsin Activity Analysis

Proteolytic activity assays was performed with some modification [8]. One g of fillet was mixed with 1 mL of aquadest and then it was separated using centrifugation at  $600 \times g$  for 10 min at 4 °C.

The supernatant was then separated again using centrifugation at  $10,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Then the extract was dissolved into 1 mL 0.1 M Tris-HCl buffer (Aplichem) pH 7.4, continued with centrifugation at  $4,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Proteolytic activity was analyzed with hemoglobin 2 % pH 2 (Oxoid) for the substrate. Substrate solution (0.5 mL) was incubated with 0.1 mL enzyme solution at  $37^\circ\text{C}$  for 10 min. Two mL Trichloro Acetic Acid (TCA) 5% was added and then filtered. The solution gained was filtrated, and then 1 mL Folin reaction was added. Final solution was read in spectrophotometry on 750 nm, blank and standard solutions (Tyrosin) were read in the same wave length.

### 3.6 Quality Characteristic Analysis

#### 3.6.1 Texture Analysis

Texture were measured using Texture analyzer TA – TX2. The probe was pressed into the fillet at a constant speed of  $2 \text{ mm} \cdot \text{s}^{-1}$  until it reached 60 % of the sample height. The maximum force obtained during compression (gf) was recorded.

#### 3.6.2 pH, Moisture Content and Salt Content

Ten g of samples were homogenized with 90 mL aquadest for 1 min. The electrode of pH meter (pH meter Hanna Instrument) was inserted into the slurry while being stirred vigorously. After stabilization, the observed value was recorded. Moisture content of samples were measured at  $105^\circ\text{C}$  according to the gravimetric test. The salt content was determined using Silver Nitrite Method.

#### 3.6.3 Protein Solubility Analysis

Protein content in supernatant was divided into two groups of experiment, i.e. set I and set II. Set I was performed with Biuret reaction and set II was using alkaline cooper sulphate reagent. Color was measured using spectrophotometer at 540 nm. Bovine serum albumin was used as the standard solution. All the analysis were run in duplicate.

### 3.7 Statistical Analysis

Group Randomly Design was used in this research and analyzed using ANOVA with significant level of 95 %. The computer software for helping this project was SPSS ver 20.

## 8.0 RESULTS AND DISCUSSION

### 8.1 Crude Cathepsin Activity

Crude cathepsin activity on raw catfish was  $0.860 \text{ U} \cdot \text{mL}^{-1}$  and it is comparable with previous experiment [5], which showed that crude cathepsin activity on cat fish after post rigor was  $0.278 \text{ U} \cdot \text{mL}^{-1}$ . The various results might be influenced by several factors such as sexual maturity level. The highest cathepsin value was reached at sexual maturity season [9]. The statistical analysis showed that smoking temperature gave significant differences for crude cathepsin activity. Based on LSD test the smoking temperature give significant different for crude cathepsin activity. The results showed that P0 and P1 did not significant different, P1 significant different with P2 and P3 but P2 did not significant different with P3 (Table 1). Reduction of crude cathepsin activity showed significant at P3 (31.98 %) from P0; 21.16 % from P0 to P2 and 13.72 % from P2 to P3.

**Table 1** Crude cathepsin activity ( $\text{U} \cdot \text{mL}^{-1}$ )

Treatment	Value
P0 (Control)	$0.860 \pm 0.06^a$
P1	$0.880 \pm 0.03^a$
P2	$0.678 \pm 0.00^b$
P3	$0.585 \pm 0.02^b$

Note: Average value of duplicate  $\pm$  standard deviation

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

Crude cathepsin activity was decreasing with the increasing of smoking temperature. The highest value of crude cathepsin activity was on smoked catfish at P1 ( $40^\circ\text{C}$  to  $50^\circ\text{C}$ ) then followed by P2 ( $60^\circ\text{C}$  to  $70^\circ\text{C}$ ) and P3 ( $80^\circ\text{C}$ ) The reduction of crude cathepsin activity may be

affected by combination of salt, heat and smoke in catfish smoking process by changing the optimum condition of enzyme to be active (Table 2). It was similar with previous experiment [5] that, the optimum condition to optimize activity of crude cathepsin were temperature, pH and metals inhibitor. The optimum temperatures were 40 °C to 50 °C and decreasing rapidly with increasing temperature in 60 °C to 70 °C. The value of optimum pH for crude cathepsin activity was recorded in the range 6 to 7, the metals such as Na could inhibit 85 % relative cathepsin activity. Gross Proteolytic Activity of smoked salmon which smoked at 20 °C to 30 °C was 0.535 mg peptides  $\cdot$ mg<sup>-1</sup> [10].

### 3.3 Texture

Based on the data that was shown at Table 2 smoking temperature and protein solubility caused the changes on smoked catfish texture. The increase of smoking temperature affected the decrease in the textural value. Heat treatments lead to denaturation on protein muscle, long heat treatment will form aggregation [11]. The textural change caused by denaturation of protein muscle, then the water soluble protein and the texture tended to semisolid gel structure resulting in the hard texture.

**Table 2** Texture (gf), pH, moisture content (wet basis %), salt content (wet basis %) and protein solubility (%) of smoked fillets catfish

Parameters	P0	P1	P2	P3
Texture	5482.43 $\pm$ 93.45 <sup>a</sup>	4591.37 $\pm$ 27.12 <sup>b</sup>	4241.93 $\pm$ 56.82 <sup>c</sup>	3881.29 $\pm$ 26.75 <sup>d</sup>
pH	7.22 $\pm$ 0.04 <sup>a</sup>	7.42 $\pm$ 0.02 <sup>b</sup>	7.72 $\pm$ 0.01 <sup>c</sup>	8.01 $\pm$ 0.04 <sup>d</sup>
Moisture content	79.66 $\pm$ 0.04 <sup>a</sup>	77.42 $\pm$ 0.02 <sup>b</sup>	74.72 $\pm$ 0.01 <sup>c</sup>	72.04 $\pm$ 0.04 <sup>d</sup>
Salt content	1.43 $\pm$ 0.10 <sup>a</sup>	2.62 $\pm$ 0.19 <sup>b</sup>	3.88 $\pm$ 0.33 <sup>c</sup>	5.83 $\pm$ 0.28 <sup>d</sup>
Protein Solubility	14.37 $\pm$ 0.42 <sup>a</sup>	11.66 $\pm$ 0.12 <sup>b</sup>	8.32 $\pm$ 0.30 <sup>c</sup>	5.80 $\pm$ 0.19 <sup>d</sup>

Note : Average value of of duplicate measurement  $\pm$  standard deviation.

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

The textural changes of smoked catfish were caused by crude cathepsin activity, even in small amount. Cathepsin activity was correlated to firmness and made textural changes on rainbow trout [12]. This enzyme was responsible for tissue degradation. The range of fibre densities from 85 fibre mm<sup>-2</sup> to 140 fibre mm<sup>-2</sup> indicates optimum "chewiness" and "firmness" in texture characteristics of smoked salmon. The high fibre densities contributed to a firmer texture in fish muscle, and the variety of fibre densities in fish was affected by sexual maturity [9].

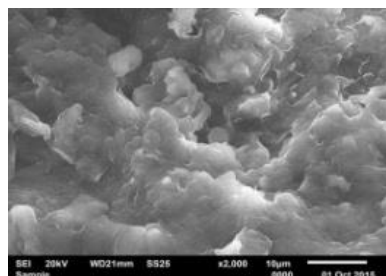
### 3.4 Scanning Electron Microscope (SEM)

Textural changes of smoked catfish were measured by Scanning Electron Microscope (SEM). SEM analysis was performed to describe structural changes in texture of smoked catfish. Figure 1(a) showed that the structure of texture smoked catfish still complex and solid. Figure 1 (b) shows aggregates in the texture of smoked catfish. Bigger aggregates were obtained from catfish which was processed in higher temperature and longer smoking time (Figure 1 (c)). Meanwhile, Figure 1 (d) shows that the texture became hardened and damaged. In the previous research, the aggregate formation increased regularly in meat heated at 60 °C, whereas meat heated at higher temperature (100 °C and 140 °C) showed dramatic increase

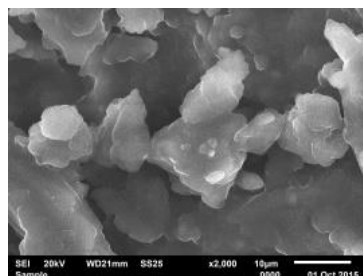
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up to 5-fold the initial level. The temperature increase promote exposure the thiol group and interior hydrophobic residues of BSA, enabling the formation of hydrogen bonds and hydrophobic interactions. This reaction and

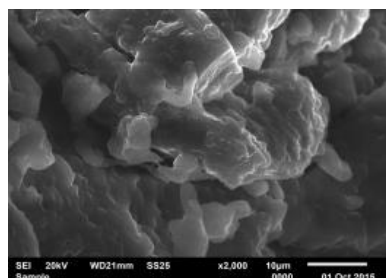
interactions promoted protein aggregation via a non-native and expanded conformational state [11,13].



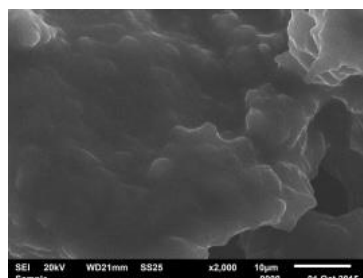
1 (a)



1 (b)



1 (c)



1 (d)

Figure 1. SEM image from smoked catfish with various treatments, i.e. (a) P0, (b) P1, (c) P2 and (d) P3



### 3.8 pH

The pH values of smoked catfish were shown in Table 2. The fresh catfish had pH values of  $7.22 \pm 0.04$ , and increased after smoking process. pH values of smoked fish from this study were 7.42 to 8.01. According to previous research on smoked stingray, pH values of smoked stingray using cocconut shell and corn cob liquid smoke were 7.30 and 8.20 respectively [6]. The changes on pH on smoked fish was affected by chemical composition of liquid smoke [14]. The value of pH is an important role to determine enzyme activity in which pH affects the ionization condition which needs the bounding between substrate and enzyme. The catalysis reaction depended on interaction between substrate with side chains amino acid which bound the active side of enzyme [15]. When pH of cathepsin was 6, cathepsin activity was  $0.271 \text{ U} \cdot \text{mL}^{-1}$ , but in pH 7 or above the value of the cathepsin activity decreased ( $0.167 \text{ U} \cdot \text{mL}^{-1}$ ) [5]. This irreversible enzyme was inactive at pH above 7 but generally highly active at acidic environment [16].

### 3.9 Moisture Content

Moisture content of smoked catfish were 72.04 % to 77.33 %, while the moisture content of raw fish was 79.66 %. Smoking process caused the reduction in the moisture content of smoked fish and, the combination of salting, pre drying and heating process evaporated the moisture in fish muscle as well. Previous research showed that moisture content of smoked milkfish processed by corn cob liquid smoke was 58.33 % and 63.37 % respectively [7].

### 3.10 Salt Content

The salt content of smoked catfish was 2.62 % to 5.83 %. There was a slight increase in the value of salt content with the increasing of smoking temperature ( $p < 0.05$ ). While smoking temperature increased, moisture evaporated and then the salt penetrated into fish flesh because of the osmosis effect from salt. In other experiment, salt content of smoked salmon were 4.0 g to 7.2 g per 100 g moisture [10]. While salt content of smoked sea bass which were processed by spray with liquid smoke for 30 min and added with NaCl (0.110 %; 0.150 %; 0.200 %; 0.220 %; 0.270 %) were 2.05 %; 4.46 %; 4.83 %; 9.40 % and 17.76 % respectively [17].

### 3.11 Protein Solubility

Protein solubility of smoked catfish decreased as a result of increasing smoking temperature, the heating process indicated the changes on protein solubility. The denaturation of protein relates to protein solubility, the tertiary or secondary structure of protein was damaged, thus, became primary structure due to heat treatments. In the primary structure, both water and salt soluble protein were released easier than in tertiary or secondary structure. The decreasing solubility of protein occurred because of increased smoking temperature. In the previous research, the protein solubility value of smoked salmon which was smoked at  $29.9^\circ\text{C}$  were significantly lower than salmon smoked at  $21.5^\circ\text{C}$  [10]. The changes of protein solubility was due to pH in which protein solubility increased at extremely acidic and alkaline environment. Previous research showed that at the extreme of pH, solubility increased to almost five times that of the original (pH 6.3), i.e.  $125.73 \pm 0.64 \text{ mg} \cdot \text{g}^{-1}$  at pH 2;  $58.92 \pm 1.10 \text{ mg} \cdot \text{g}^{-1}$  at pH 4;  $21.42 \pm 0.5 \text{ mg} \cdot \text{g}^{-1}$  at pH 6;  $44.76 \pm 0.95 \text{ mg} \cdot \text{g}^{-1}$  at pH 8 and  $122.85 \pm 1.2 \text{ mg} \cdot \text{g}^{-1}$  at pH 12 [18]. Another study showed that minimum protein solubility that called isoelectric point, in raw or cooked samples exhibited at pH 5 to 6. Protein solubility decreased with increasing pH to isoelectric point then increased again to high pH [19].

## 4.0 CONCLUSION

Inceasing smoking temperature could inhibit the crude cathepsin enzyme activity and reduced the texture, moisture content, and protein solubility of smoked catfish. The information from this study could be a reference to produce good quality smoked fish in particular protein nutritive aspect.

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Demikian yang dapat saya sampaikan. Saya ucapkan terima kasih atas perhatiannya.

Salam,

Monika





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Yahoo Mail - Koreksian naskah a.n. Fronthea Swastawati (2016.02.04)

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Dari: Monika Nur Utami Prihastyanti, S.TP, M.Nat.Sc (monika.nur@machung.ac.id)

Kepada: fronthea\_thp@yahoo.co.id; fronthea\_thp@undip.ac.id

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Terima kasih atas perhatiannya.

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**CRUDE CATHEPSIN ACTIVITY AND QUALITY  
CHARACTERISTIC OF SMOKED CATFISH  
(PANGASIU PANGASIU) PROCESSED BY  
DIFFERENT SMOKING TEMPERATURE**

## Article history

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Fronthea Swastawati<sup>a\*</sup>, Ahmad Ni'matullah Al Baarri<sup>b</sup>, Tri  
Winarni Agustini<sup>a</sup>, Eko Nurcahya Dewi<sup>a</sup>, Ima Wijayanti<sup>a</sup>,  
Dwi Yanuar Budi Prasetyo<sup>c</sup>

.....

<sup>a</sup>Laboratory of Fish Processing Technology, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Prof Soedarto, SH, Tembalang, Semarang – 50275,  
Indonesia

\*Corresponding author

fronthea\_thp@undip.ac.id

Tel. +6281225191989

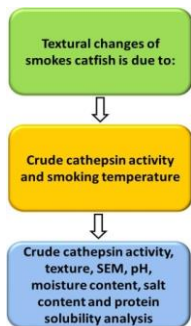
<sup>b</sup>Laboratory of Chemistry and Food Nutrition, Faculty of  
Animal and Agriculture, Diponegoro University, Semarang  
– 50275, Indonesia

<sup>c</sup>Magister Coastal Resource Management, Faculty of  
Fisheries and Marine Science, Diponegoro University, Jl.  
Imam Bardjo, SH, Semarang – 50241, Indonesia

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## Graphical abstract



## Abstract

The aim of this research were to investigate the effect of smoking temperature towards crude cathepsin activity and quality characteristic of smoked catfish (*Pangasius pangasius*). Different smoking temperature had significant effect ( $p < 0.05$ ) on crude cathepsin activity, texture, pH, moisture content, salt content and protein solubility. The significant decreasing (30.13 %) of crude cathepsin activity at P3 (80 °C) from P1 (40 °C to 50 °C). Many factors were correlated to the textural changes of smoked catfish such as changes of crude cathepsin activity, reduction of protein solubility and pH value.

**Keywords :** Catfish (*Pangasius pangasius*), crude cathepsin activity, quality characteristic, smoking temperature

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## 9.0 INTRODUCTION

Catfish (*Pangasius pangasius* (Hamilton, 1882)) is one of the economic aquaculture fish species that can be found in Indonesia's waters. Its production was increasing by 37 % during 2008 to 2012 [1]. In terms of its nutrition aspect, catfish contains about 35 % of protein and 10.1 % of lipid with dominant fatty acids profile were lauric acid (13.36 %), palmitic acid (26.15 %), oleic acid (46.07 %) and stearic acid (14.40 %) which contribute to human health [2]. Texture is an important quality characteristic and acceptability factor for consumer. The texture of catfish is influenced by several factors such as size, age, macro nutrients composition (protein and lipid content), handling and processing condition. Catfish is one of many aquatic products which is highly perishable, in which after the post mortem the enzymatic decomposition rapidly breaks down the texture of fish components like myofibril and connective tissue. [3], cathepsin enzyme activity could change the structure and firmness. Cathepsin B, L and D were able to decrease  $\alpha$ -actinin which is responsible to the fish firmness. A significant correlation was observed between enzymatic activity of cathepsin B and L and muscle degradation of Atlantic salmon [4].

Smoking process could inhibit the enzymatic decomposition. Brinning, pre drying, chemical composition of smoke and heating in smoking process could change the optimum condition of cathepsin to be active. Cathepsin needs a certain

condition to optimize their activity, such as temperature, pH, substrate concentration and the presence of metal inhibitor [5]. Nowadays smoking is not only a preservative method but it also gives a specific flavour and taste on smoked fish to increase the consumer acceptability. This role could be obtained using liquid smoke, which is easier to apply and is environmentally safe [6]. The aim of this research was to investigate the effects of smoking temperature to crude cathepsin activity which affect textural changes and quality characteristic of smoked catfish.

## 10.0 EXPERIMENTAL

### 2.1 Smoking Process

Smoking method of catfish was performed with some modification [7]. Fillets of catfish were separated into four groups. Each group dipped into 5 % brine and 5 % liquid smoke for 30 min. Pre-drying in room temperature for about 60 min and then smoked in the electrical oven at 40 °C to 50 °C (P1), 60 °C to 70 °C (P2) and 80 °C (P3) for 1 h each. Then it was chilled in the room temperature and then packed with polyethylene bag before analysis in the laboratory.

### 3.8 Crude Cathepsin Activity Analysis

Proteolytic activity assays was performed with some modification [8]. The sample was prepared by mixing 1 g of fillet with 1 mL of aquadest and

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then it was separated using centrifugation at 2 315 rpm (1 rpm = 1/60 Hz) for 10 min at 4 °C. The supernatant was then separated again using centrifugation at 5 976 rpm for 10 min at 4 °C. Then the extract was dissolved into 1 mL 0,1 M Tris-HCl buffer (Aplichem) pH 7.4, continued with centrifugation at 9 449 rpm for 10 min at 4 °C. Proteolytic activity was analyzed with hemoglobin 2 % pH 2 (Oxoid) for the substrate. Substrate solution (0.5 mL) was incubated with 0.1 mL enzyme solution at 37 °C for 10 min. Subsequently, 2 mL Trichloro Acetic Acid (TCA) 5 % was added and then filtered. The solution gained was filtrated, and then 1 mL Folin reaction was added. Final solution was read in spectrophotometry on 750 nm, blank and standard solutions (Tirosin) were read in the same wave length.

### 3.9 Quality Characteristic Analysis

#### 3.9.1 Texture Analysis

Texture were measured using Texture analyzer TA – TX2. The probe was pressed into the fillet at a constant speed of 2 mm · s<sup>-1</sup> until it reached 60 % of the sample height. The maximum force obtained during compression (gf) was recorded.

#### 3.9.2 pH, Moisture Content and Salt Content

For analysis preparation, 10 g of samples were homogenized with 90 mL aquadest for 1 min. The electrode of pH meter (pH meter Hanna Instrument) was inserted into the slurry while being stirred vigorously. After stabilization, the observed value was recorded. Moisture content of samples were measured at 105 °C according to the gravimetric test. The salt content was determined using Silver Nitrite Method.

#### 3.9.3 Protein Solubility Analysis

Protein content in supernatant was divided into two groups of experiment, i.e. set I and set II. Set I was performed with Biuret reaction and set II was using alkaline cooper sulphate reagent. Color was measured using spectrophotometer at 540 nm. Bovine serum albumin was used as the standard solution. All the analysis were run in duplicate.

### 3.10 Statistical Analysis

Randomized Block Design was used in this research and analyzed using ANOVA with significant level of 95 %. The computer software for helping this project was SPSS ver 20.

## 11.0 RESULTS AND DISCUSSION

### 11.1 Crude Cathepsin Activity

Crude cathepsin activity on raw catfish was 0.860 U · mL<sup>-1</sup> and it is comparable with previous experiment [5], which showed that crude cathepsin activity on cat fish after post rigor was 0.278 U · mL<sup>-1</sup>. The various results might be influenced by several factors such as sexual maturity level. The highest cathepsin value was reached at sexual maturity season [9]. The statistical analysis showed that smoking temperature gave significant differences for crude cathepsin activity. Based on LSD test the smoking temperature give significant different for crude cathepsin activity. The results showed that P0 and P1 did not significant different, P1 significant different with P2 and P3 but P2 did not significant different with P3 (Table 1). Reduction of crude cathepsin activity showed significant at P3 (31.98 %) from P0; 21.16 % from P0 to P2 and 13.72 % from P2 to P3.

**Table 1** Crude cathepsin activity (U · mL<sup>-1</sup>)

Treatment	Value
P0 (Control)	0.860 ± 0.06 <sup>a</sup>
P1	0.880 ± 0.03 <sup>a</sup>
P2	0.678 ± 0.00 <sup>b</sup>
P3	0.585 ± 0.02 <sup>b</sup>

Note: Average value of duplicate ± standard deviation

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

Crude cathepsin activity was decreasing with the increasing of smoking temperature. The highest value of crude cathepsin activity was on smoked catfish at P1 (40 °C to 50 °C) then

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followed by P2 (60 °C to 70 °C) and P3 (80 °C). The reduction of crude cathepsin activity may be affected by combination of salt, heat and smoke in catfish smoking process by changing the optimum condition of enzyme to be active (Table 2). It was similar with previous experiment [5] that, the optimum condition to optimize activity of crude cathepsin were temperature, pH and metals inhibitor. The optimum temperatures were 40 °C to 50 °C and decreasing rapidly with increasing temperature in 60 °C to 70 °C. The value of optimum pH for crude cathepsin activity was recorded in the range 6 to 7, the metals such as Na could inhibit 85 % relative cathepsin activity. Gross Proteolytic Activity of smoked salmon which smoked at 20 °C to 30 °C was 0.535 mg peptides  $\cdot$ mg<sup>-1</sup> [10].

### 3.4 Texture

Based on the data that was shown at Table 2 smoking temperature and protein solubility caused the changes on smoked catfish texture. The increase of smoking temperature affected the decrease in the textural value. Heat treatments lead to denaturation on protein muscle, long heat treatment will form aggregation [11]. The textural change caused by denaturation of protein muscle, then the water soluble protein and the texture tended to semisolid gel structure resulting in the hard texture.

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**Table 2** Texture (gf), pH, moisture content (wet basis %), salt content (wet basis %) and protein solubility (%) of smoked fillets catfish

Parameters	P0	P1	P2	P3
Texture	5482.43 ± 93.45 <sup>a</sup>	4591.37 ± 27.12 <sup>b</sup>	4241.93 ± 56.82 <sup>c</sup>	3881.29 ± 26.75 <sup>d</sup>
pH	7.22 ± 0.04 <sup>a</sup>	7.42 ± 0.02 <sup>b</sup>	7.72 ± 0.01 <sup>c</sup>	8.01 ± 0.04 <sup>d</sup>
Moisture content	79.66 ± 0.04 <sup>a</sup>	77.42 ± 0.02 <sup>b</sup>	74.72 ± 0.01 <sup>c</sup>	72.04 ± 0.04 <sup>d</sup>
Salt content	1.43 ± 0.10 <sup>a</sup>	2.62 ± 0.19 <sup>b</sup>	3.88 ± 0.33 <sup>c</sup>	5.83 ± 0.28 <sup>d</sup>
Protein Solubility	14.37 ± 0.42 <sup>a</sup>	11.66 ± 0.12 <sup>b</sup>	8.32 ± 0.30 <sup>c</sup>	5.80 ± 0.19 <sup>d</sup>

Note : Average value of of duplicate measurement ± standard deviation.

Value following with same small superscript letters were no significantly different ( $p > 0.05$ ).

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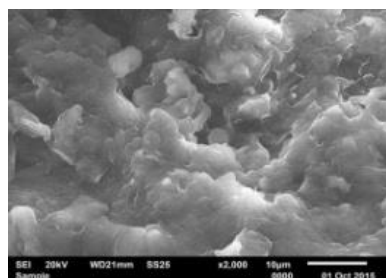
The textural changes of smoked catfish were caused by crude cathepsin activity, even in small amount. Cathepsin activity was correlated to firmness and made textural changes on rainbow trout [12]. This enzyme was responsible for tissue degradation. The range of fibre densities from 85 fibre mm<sup>-2</sup> to 140 fibre mm<sup>-2</sup> indicates optimum "chewiness" and "firmness" in texture characteristics of smoked salmon. The high fibre densities contributed to a firmer texture in fish muscle, and the variety of fibre densities in fish was affected by sexual maturity [9].

### 3.5 Scanning Electron Microscope (SEM)

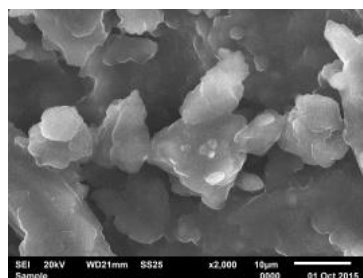
Textural changes of smoked catfish were measured by Scanning Electron Microscope (SEM). SEM analysis was performed to describe structural changes in texture of smoked catfish. Figure 1(a) showed that the structure of texture smoked catfish still complex and solid. Figure 1 (b) shows aggregates in the texture of smoked catfish. Bigger aggregates were obtained from catfish which was processed in higher temperature and longer smoking time (Figure 1 (c)). Meanwhile, Figure 1 (d) shows that the texture became hardened and damaged. In the previous research, the aggregate formation increased regularly in meat heated at 60 °C, whereas meat heated at higher temperature

(100 °C and 140 °C) showed dramatic increase up to 5-fold the initial level. The temperature increase promote exposure the thiol group and interior hydrophobic residues of BSA, enabling the formation of hydrogen bonds and hydrophobic interactions. This reaction and

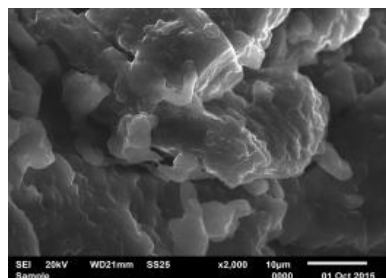
interactions promoted protein aggregation via a non-native and expanded conformational state [11,13].



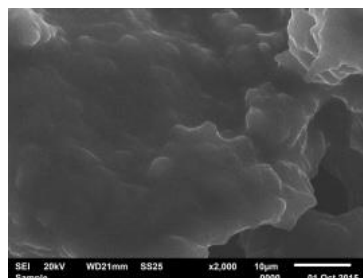
1 (a)



1 (b)



1 (c)



1 (d)

Figure 1. SEM image from smoked catfish with various treatments, i.e. (a) P0, (b) P1, (c) P2 and (d) P3

### 3.12pH

The pH values of smoked catfish were shown in Table 2. The fresh catfish had pH values of  $7.22 \pm 0.04$ , and increased after smoking process. pH values of smoked fish from this study were 7.42 to 8.01. According to previous research on smoked stingray, pH values of smoked stingray using cocconut shell and corn cob liquid smoke were 7.30 and 8.20 respectively [6]. The changes on pH on smoked fish was affected by chemical composition of liquid smoke [14]. The value of pH is an important role to determine enzyme activity in which pH affects the ionization condition which needs the bounding between substrate and enzyme. The catalysis reaction depended on interaction between substrate with side chains amino acid which bound the active side of enzyme [15]. When pH of cathepsin was 6, cathepsin activity was  $0.271 \mu \cdot \text{mL}^{-1}$ , but in pH 7 or above the value of the cathepsin activity decreased ( $0.167 \mu \cdot \text{mL}^{-1}$ ) [5]. This irreversible enzyme was inactive at pH above 7 but generally highly active at acidic environment [16].

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### 3.13Moisture Content

Moisture content of smoked catfish were 72.04 % to 77.33 %, while the moisture content of raw fish was 79.66 %. Smoking process caused the reduction in the moisture content of smoked fish and, the combination of salting, pre drying and heating process evaporated the moisture in fish muscle as well. Previous research showed that moisture content of smoked milkfish processed by corn cob liquid smoke was 58.33 % and 63.37 % respectively [7].

### 3.14 Salt Content

The salt content of smoked catfish was 2.62 % to 5.83 %. There was a slight increase in the value of salt content with the increasing of smoking temperature ( $p < 0.05$ ). While smoking temperature increased, moisture evaporated and then the salt penetrated into fish flesh because of the osmosis effect from salt. In other experiment, salt content of smoked salmon were 4.0 g to 7.2 g per 100 g moisture [10]. While salt content of smoked sea bass which were processed by spray with liquid smoke for 30 min and added with NaCl (0.110 %; 0.150 %; 0.200 %; 0.220 %; 0.270 %) were 2.05 %; 4.46 %; 4.83 %; 9.40 % and 17.76 % respectively [17].

### 3.15 Protein Solubility

Protein solubility of smoked catfish decreased as a result of increasing smoking temperature, the heating process indicated the changes on protein solubility. The denaturation of protein relates to protein solubility, the tertiary or secondary structure of protein was damaged, thus, became primary structure due to heat treatments. In the primary structure, both water and salt soluble protein were released easier than in tertiary or secondary structure. The decreasing solubility of protein occurred because of increased smoking temperature. In the previous research, the protein solubility value of smoked salmon which was smoked at  $29.9^\circ\text{C}$  were significantly lower than salmon smoked at  $21.5^\circ\text{C}$  [10]. The changes of protein solubility was due to pH in which protein solubility increased at extremely acidic and alkaline environment. Previous research showed that at the extreme of pH, solubility increased to almost five times that of the original (pH 6.3), i.e.  $125.73 \pm 0.64 \text{ mg} \cdot \text{g}^{-1}$  at pH 2;  $58.92 \pm 1.10 \text{ mg} \cdot \text{g}^{-1}$  at pH 4;  $21.42 \pm 0.5 \text{ mg} \cdot \text{g}^{-1}$  at pH 6;  $44.76 \pm 0.95 \text{ mg} \cdot \text{g}^{-1}$  at pH 8 and  $122.85 \pm 1.2 \text{ mg} \cdot \text{g}^{-1}$  at pH 12 [18]. Another study showed that minimum protein solubility that called isoelectric point, in raw or cooked samples exhibited at pH 5 to 6. Protein solubility decreased with increasing pH to isoelectric point then increased again to high pH [19].

## 4.0 CONCLUSION

Inceasing smoking temperature could inhibit the crude cathepsin enzyme activity and reduced the texture, moisture content, and protein solubility of smoked catfish. The information from this study could be a reference to produce good quality smoked fish in particular protein nutritive aspect.

### Acknowledgements

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Salam,

Monika



Status Naskah JT-UTM (2016.02.26, 11.00 am).xlsx  
68.3kB

10/20/2020

Yahoo Mail - FW: Submit manuscripts from ISAPPROSH-2015

FW: Submit manuscripts from ISAPPROSH-2015

Dari: roy hendroko (roy\_hendroko@hotmail.com)

Kepada: fronthea\_thp@yahoo.co.id

Tanggal: Rabu, 2 Maret 2016 12.14 WIB

Ysh Bu Thea

Di bawah adalah email dari JT-UTM. Mohon Ibu agar mempersiapkan dana sesuai kalimat di bawah yang **warna merah**.  
Maturwun.

Wass  
RHS

Date: Wed, 2 Mar 2016 12:44:28 +0800  
Subject: Re: Submit manuscripts from ISAPPROSH-2015  
From: norazmans@utm.my  
To: roy\_hendroko@hotmail.com  
CC: piza@utm.my; hairunnisa@utm.my

**Dear Roy,****We have downloaded from WeTransfer the following files :**

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**Thank you.**

F

10/20/2020

Yahoo Mail - Revisi Tahap II Manuskrip ISAPPROSH2 untuk JT-UTM

## Revisi Tahap II Manuskrip ISAPPROSH2 untuk JT-UTM

Dari: roy hendroko (roy\_hendroko@hotmail.com)

Kepada: rasyid.romadhony@gmail.com; susilowati\_ragil@yahoo.com; istiyanto\_samidjan@yahoo.com;  
Latifahubawa2004@yahoo.com; fronthea\_thp@yahoo.co.id; s\_harsono@yahoo.com; suryantidr@gmail.com;  
tagustini@yahoo.com

Cc: fronthea\_thp@yahoo.co.id; laras\_rianingsih@yahoo.com; monika.nur@machung.ac.id; rosita.dwi@machung.ac.id;  
katarinapurnomo@gmail.com

Tanggal: Jumat, 8 April 2016 17:35 WIB

## Ysh Para Author

Alhamdulillah, puji syukur publikasi JT-UTM telah sampai tahap prof-read oleh editorial board JT-UTM. Namun masih terdapat sejumlah permintaan revisi oleh mereka. Mbak Monika telah mengirim email ke Bapak dan Ibu tentang permintaan revisi manuskrip Anda. Saya mohon kerja sama Bapak dan Ibu untuk berkenan mematuhi tengat waktu yang telah kami menetapkan. Batas waktu perbaikan adalah **Senin, 11 April 2016, jam 12.00 WIB** telah diterima di **email Mbak Monika dengan cc ke Roy Hendroko**.

Apabila ada pertanyaan tentang instruksi revisi tsb, mohon dengan segera menghubungi Mbak Monika melalui email, dan/atau SMS-WA di 082139507456.

Kepada Bu Laras dan/atau Bu Thea, mohon bantuan berkenan untuk SMS atau WA para author di atas untuk *gentle reminder*

Semoga tahapan ini dapat kita melewatinya dengan sukses. Maturwun

Salam dan doa  
RHS

10/21/2020

Yahoo Mail - JT-UTM Vol. 78 No4-2

## JT-UTM Vol. 78 No4-2

Dari: roy hendroko (roy\_hendroko@hotmail.com)

Kepada: frontheta\_thp@yahoo.co.id; laras\_rianingsih@yahoo.com; lukita\_anandito@yahoo.com; ekothp@live.undip.ac.id

Cc: praptiningsih.ga@gmail.com; monika.nur@machung.ac.id; katarinapurnomo@gmail.com; rosita.dwi@machung.ac.id

Tanggal: Senin, 18 April 2016 10.24 WIB

Ysh Para Rekan.

Mohon doa agar proef Vol 78 No 4-2 yang saya unggah dapat segera on-line. Insya Allah dapat memenuhi deadline Rektor UNDIP, yakni sebelum 21 April 2016.

Maturwun.

Wass

RHS



Vol 78, No 4-2\_ Sustaining Fish Processing Industry to Support Global Maritime Axis.pdf  
307.7kB

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Fw: Notification of Publication Jurnal Teknologi (Sciences & Eng.) Special Issue: Sustaining Fish Processing Industry to Support Global Maritime Axis

Dari: roy hendroko (roy\_hendroko@hotmail.com)

Kepada: frontheta\_thp@yahoo.co.id; laras\_rianingsih@yahoo.com; lukita\_anandito@yahoo.com; ekothp@live.undip.ac.id

Cc: monika.nur@machung.ac.id; katarinapurnomo@gmail.com; rosita.dwi@machung.ac.id; tatas.brotosudarmo@machung.ac.id; chatief.kunjaya@machung.ac.id

Tanggal: Senin, 18 April 2016 12.50 WIB

Ysh Ibu Dr. Fronthea Swastawati  
UNDIP

Dengan ucapan Alhamdulillah, saya dengan gembira dan bangga melaporkan hasil kerja kita telah on-line di Jurnal-Teknologi Vol. 78 No 4-2, seperti forward email di bawah ini, dan juga atach email. Puji syukur pula kami (saya + Bu Prapti dan Tim MRCPP) mampu menyelesaikan sebelum deadline tanggal Rektor UNDIP. Hari ini tanggal 18 April 2016, sedang tengat waktu adalah Hari Kartini, 21 April 2016.

Puji syukur, kita dapat mengatasi sejumlah "kendala birokrasi" di Malaysia. Seperti diketahui semula kita diberi jatah Vol. 78 No 3. Tetapi dengan alasan manuskrip ISAPPROSH membutuhkan sejumlah revisi maka Vol jatah kita "diberikan/ dipindahkan" ke conference/ issue yang lain. Namun berkat bantuan dan kerja keras Mbak Laras + Mbak Lukita yang membantu "mengejar" revisi 20 author, kita mampu menyelesaikan revisi maju dibanding skedul. Demikian pula di revisi ke-2 terhadap 10 author, Mbak Monika, Mbak Rosita (bahkan Mbak Katrin di jelang pemberkatan pernikahannya) penuh semangat menyelesaikan revisi lebih maju dari skedul yang ditetapkan oleh editor JT-UTM.

Seperti Anda melihat di sini (<http://www.jurnalteknologi.utm.my/index.php/jurnalteknologi/issue/archive>) sampai hari ini (18 April 2016) ternyata Vol 78 No 2, Vol. 78 No 3 dan Vol 78 No. 4 yang mereka mencadangkan untuk sejumlah conference/ issue tersebut TIDAK/ Belum mampu submit untuk online.

Maturwun atas segala kerjasama yang baik sehingga JT-UTM dapat terselesaikan. Namun kita masih mempunyai PR tentang manuskrip ISAPPROSH yang lain.

Allah memberkati karya kita.  
Roy Hendroko Setyobudi

From: HAPIZAH BINTI MOHMAD@MOHAMAD TNCPi <piza@utm.my>

Sent: Monday, April 18, 2016 10:54 AM

To: roy hendroko

Cc: ROSLI BIN MD. ILLIAS TNCPi; KASSIM BIN THUKIMAN TNCPi; UNGKU HALIZA BT UNGKU ZAHAR TNCPi; MD RAZIB BIN KARNO PSZ

Subject: Notification of Publication Jurnal Teknologi (Sciences & Eng.) Special Issue: Sustaining Fish Processing Industry to Support Global Maritime Axis

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Thank you

**Hapizah Mohamad**  
Publication Officer (Journal)

**Penerbit UTM Press**  
Universiti Teknologi Malaysia  
81310 UTM Johor Bahru  
Johor, Malaysia

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