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CRUDE CATHEPSIN ACTIVITY AND QUALITY CHARACTERISTIC OF SMOKED CATFISH [*Pangasius pangasius* (Hamilton, 1882)] PROCESSED BY DIFFERENT SMOKING TEMPERATURE

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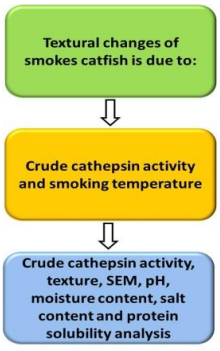
Graphical abstract



Abstract

The aim of this research was to investigate the effect of smoking temperature towards crude cathepsin activity and quality characteristic of smoked catfish [*Pangasius pangasius* (Hamilton, 1882)]. 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 was 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* (Hamilton, 1882)], crude cathepsin activity, quality characteristic, smoking temperature

1.0 INTRODUCTION

Catfish [*Pangasius pangasius* (Hamilton, 1882)] is one of the economic aquaculture fish species 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 nutrient composition (protein as well as 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 a-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. Cathepsinne need 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 investigated the effects of smoking temperature to crude cathepsin activity which affect textural changes and quality characteristic of smoked catfish.

2.0 MATERIAL AND METHOD

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 poliethylene bag before analysis in the laboratory.

2.2 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 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 Acetid Acid (TCA) 5 % was added and then filltered. 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.

2.3 Quality Characteristic Analysis

2.3.1 Texture Analysis

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

2.3.2 pH, Moisture Content and Salt Content

For analysis preparation, 10 g of samples were homogenized with 90 mL of distilled water 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 was measured at 105 °C according to the gravimetric test. The salt content was determined using Silver Nitrite Method.

2.3.3 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.

2.4 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.

3.0 RESULTS AND DISCUSSION

3.1 Crude Cathepsin Activity

Crude cathepsin activity on raw catfish was 0.860 U · mL^{-1} and it is comparable with previous experiment [5], which showed that crude chatepsin activity on cat fish after post rigor was 0.278 U mL^{-1} (1 U = 1/60 micro katal). 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 there was no significant difference between treatment P0 and P1 as well as between treatment P2 and P3. Result showed significant

difference between treatment P1 and P2 (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^{-1})

Treatment	Value
P0 (Control)	0.860 ± 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 ± standard deviaton
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 optimalize 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 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.

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^{-2} to 140 fibre 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 [9].

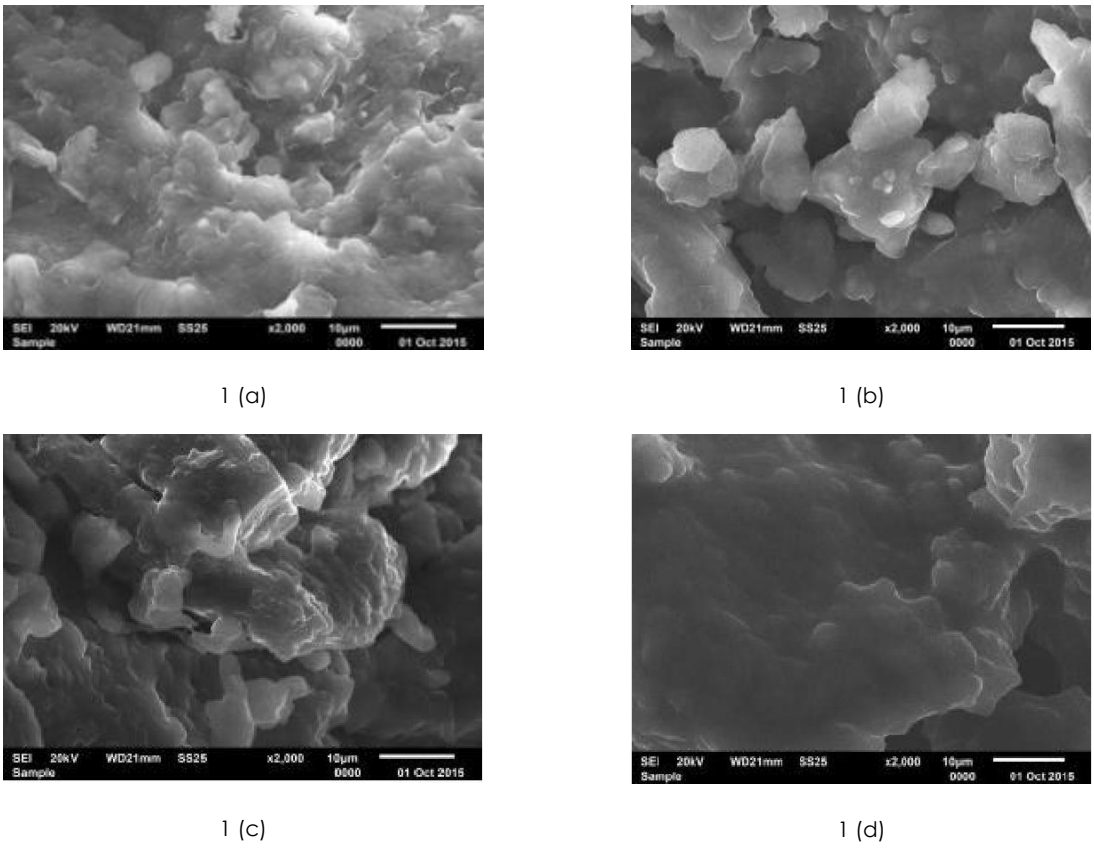
Table 2 Texture (gf), pH, moisture content (wet basis %), salt content (wet basis %) and protein solubility (%) of smoked fillets catfish

Parameters	P0	P1	P2	P3
Texture	5482.43 ± 93.45^{a}	4591.37 ± 27.12^{b}	4241.93 ± 56.82^{c}	3881.29 ± 26.75^{d}
pH	7.22 ± 0.04^{a}	7.42 ± 0.02^{b}	7.72 ± 0.01^{c}	$8,01 \pm 0.04^{d}$
Moisture content	79.66 ± 0.04^{a}	77.42 ± 0.02^{b}	74.72 ± 0.01^{c}	72.04 ± 0.04^{d}
Salt content	1.43 ± 0.10^{a}	2.62 ± 0.19^{b}	3.88 ± 0.33^{c}	5.83 ± 0.28^{d}
Protein Solubility	14.37 ± 0.42^{a}	11.66 ± 0.12^{b}	8.32 ± 0.30^{c}	5.80 ± 0.19^{d}

Note : Average value of of duplicate measurement ± standard deviaton.
Value following with same small superscript letters were no significantly different ($p > 0.05$).

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) 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

3.4 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 to 8.01. According to previous research on smoked stingray, pH values of smoked stingray using coconut shell and corn cob liquid smoke were 7.30 and 8.20 respectively [6]. The changes on pH on smoked fish were 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 U mL^{-1}, but in pH 7 or above the value of the cathepsin activity decreased (0.167 U mL^{-1}) [5]. This irreversible enzyme was inactive at pH above 7 but generally highly active at acidic environment [16].

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 [17].

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, 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 °C were significantly lower than salmon smoked at 21.5 °C [10]. The changes of protein solubility were 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 ± 0.64 mg g^{-1} at pH 2; 58.92 ± 1.10 mg g^{-1} at pH 4; 21.42 ± 0.5 mg g^{-1} at pH 6; 44.76 ± 0.95 mg g^{-1} at pH 8 and 122.85 ± 1.2 mg 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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