ACE inhibition and antioxidant activity of different part of Channa striata prepared by various cooking method

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Abstract. Channa striata (snakehead) extract has been known possessing positive activity, one of which is the ability to inhibit Angiotensin Converting Enzyme (ACE) activity in vitro. Aims of this study were to determine the effect of cooking and parts of C. striata, i.e. meat/fillet, gonad, skin, gill against the ACE inhibition activity and antioxidant activity in vitro. Heat processing methods used were direct boiling and indirect boiling and steamed at 100 °C for 10 min. ACE inhibition activity was analyzed using hippuryl-L-histidyl-L-leucine (HHL) as substrate and antioxidant activity was analyzed using DPPH method. The result shows that the higher the concentration of the extract (5 %, 20 %, 35 % and 50 %), the higher the antioxidant activity. The highest antioxidant activity was shown by gonad followed by meat extract, skin, and gill. Cooking treatment affected antioxidant activity, being the detrimental treatment were steam and direct boiling. The egg/gonad of C. striata showed the highest capability to inhibit ACE activity followed by meat/fillet, gill and skin. In concentration of 10 mg, extract of C. striata gonad was comparable to captopril, a commercial hypertension drug. While uncooked fillet showed the highest ACE inhibition activity followed by indirect boiling, direct boiling and steaming.

1. Introduction
Fish and seafood, in general, is well known as nutritious food containing high quality of protein and lipid important for health. Regular consumption of fish is important for children to combat malnutrition and for efficient prevention of several degenerative diseases for adult people. Snakehead or Channa striata is an indigenous freshwater fish species in Indonesia, and well known to have high nutritional value, i.e. excellence amino acid and lipid composition [1, 2, 3]. Rich in amino acid glycine and lipid arachidonic acid, C. striata, well known as haruan in Malaysia and ikan gabus in Indonesia, has been promoted to be used for postoperative wound healing, anti-inflammatory, antinociceptive, platelet aggregation, lowering blood glucose as well as mild antimicrobial and antifungal properties [4, 5, 6, 7]. C. striata also had been reported to have peptide released when the meat was hydrolyzed biologically, which was active against ACE or hypertension [8, 9].

Hypertension is becoming a global health concern including Indonesia. Hypertension or high blood pressure is responsible for one-quarter of all deaths in the world [10]. The World Health Organization reported that more than 60 % of strokes and 50 % of heart attacks are caused by high blood pressure [11], causing economic loss due to high-cost lifetime treatment. The Centre for Disease Control and Prevention (CDC) the USA has estimated that hypertension-related costs reached $ 76.6 billion in 2010 [11]. Hypertension or high blood pressure is related to Angiotensin I-converting enzyme (ACE; peptidylpeptide hydrolase, EC 3.4.15.1) activity that plays an important role in the regulation of blood...
pressure as well as in cardiovascular function [12]. ACE provides the powerful vasoconstrictor angiotensin II and removes the C-terminal dipeptide from the lysate of the precursor decapeptide angiotensin I. Increasing vasoconstriction and the development of high blood pressure were therefore as consequence the presence of high-level ACE in the body of patients. Extract of C. striata meat without hydrolysis was positively active against ACE in vitro, suggesting that C. striata extract was potentially used to combat hypertension [3].

In recent years, peptides have received more attention due to their activity against hypertension by preventing the ACE activity. Bioactive peptides derived from food proteins are safe compared to the synthetic hypertension drug but milder activity [13]. Peptides are defined as short-chain protein molecules (usually < 20 amino acid residues), therefore, in food rich in protein such as fish, peptides can be processed by hydrolyzing the protein. Bioactive peptides from biologically hydrolyzed snakehead fish myofibrillar protein and sarcoplasmic protein have been isolated [9, 14]. Using commercial protease, alcalase, two novel ACE inhibitor peptides LYPPP and YSMYPP with IC50 values of 1.3 and 2.8 mM were identified, respectively. Peptides from fish and shellfish have acknowledged lately as sources of natural antioxidant as well [15].

Antioxidants play an important role in human health due to their capability in scavenging the reactive oxygen species (ROS) such as superoxide anion (O2−), hydroxyl radical (OH) and hydrogen peroxide (H2O2) that are normally produced in living organisms during metabolism. The antioxidant has been reported to have an important role in preventing cell injury [16]. ROS has been implicated taking a role in human disorders such as in diabetes, hypertension, atherosclerotic cardiovascular disease and cancer [13]. Most of the natural antioxidants reported were mostly non-protein compounds in plants.

When consuming C. striata for maximal health benefit, people do cooking usually by direct heating such as steaming. Cooking was also intended to enhance flavor and taste of food and inactivate pathogenic microorganisms. The objective of this work was to obtain the best tissue part of C. striata and the best cooking strategy to obtain C. striata extract with maximal ACE inhibition and antioxidant activity.

2. Material and Methods

2.1. Sample preparation and extraction
Wild Channa striata of 15 kg (average weight and the total length of 100–350 g and 28–34 cm, respectively) were obtained from lakes surrounding Parung, Bogor, West Java, Indonesia, and was life transported to the laboratory in a plastic bag. The fish was killed by icing them for 15 min as soon as the fish in the laboratory. Filleting was conducted to obtain fish flesh, meanwhile, gonad/egg, gill, and skin were separated. All preparation was conducted through cold chain system, and the meat, gonad, gill, and skin were frozen at -20 °C before following day’s extraction.

Extraction was following Falkenberg et al. [17] with modification. Thawed meat, gill, egg/gonad and skin of Channa striata were minced in a food processor and 20 g of the meat was added with 60 mL of distilled water. The suspension was homogenized with Ultraturrax T25 (IKA Labortechnich) using the speed of 13,500 rpm for 5 min in ice. The suspension was centrifuged for 15 min at 10,000 g, 4 °C. The supernatant was used for treatment and analysis. The experiment was conducted in triplicates.

Fillet of Channa striata was heat processed by 1) direct boiling 10 min (in water), 2) indirect boiling 10 min (using a water bath) and 3) steaming at 100 °C for 10 min. Extraction was conducted as previously mention after heat treatment.

2.2. Protein content analysis
Protein content was analyzed using [18] against a standard curve of Bovine Serum Albumin (BSA) standard.
2.3. Measurement of antioxidant activity

Antioxidant activity was assessed using DPPH radical scavenging activity [19] with modification. Sample extracts at a concentration of 30 μg·mL⁻¹ were tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl DPPH method. DPPH (Merck) solution was prepared by solubilizing 3 mg DPPH in 5 mL methanol. Extract of 160 μL was added with 40 μL DPPH in a 96 microplate well and then incubated for 30 min in a dark room. The absorbance was read using microplate reader at 517 nm. Vitamin C was used (10 μg·mL⁻¹) as a control.

2.4. Measurement of ACE inhibitor activity

Inhibition activity of Angiotensin-I Converting Enzyme (ACE) was determined based on the formation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) measured by UV spectrophotometer [20]. Sample (50 μL, 15 mg·mL⁻¹) added with 125 μL substrate buffer (containing 7.6 mM N-hippuric-his-leu hydrate and 608 mM NaCl in 10 mL borate buffer pH 8.3), and added with 15 μL BSA (10 mg·mL⁻¹). The mixture then incubated at 37 °C for 15 min using shaker water bath. The reaction started by addition of 50 μL ACE 50 μU·mL⁻¹ and incubated for 30 min. The reaction was stopped by addition of 200 μL HCl 1 N, and after mixed using vortex, 1,140 μL ethyl acetate was added to extract hippuric acid. The mixture was centrifugated at 10,000 g for 10 min. The supernatant (1,000 μL) was dried (oven 95 °C for 75 min). After solubilized with 1,000 μL aquabides, the absorbance was measured at 228 nm using spectrophotometer UV-Vis. The positive control used was captopril, a hypertension drug. ACE inhibition (%) was calculated as:

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\frac{[(K-BK)-(S-BS)]}{(K-BK)} \times 100
\]

Where K = absorbance of control; BK = absorbance of control blank; S = absorbance of sample; BS = absorbance of sample blank

All analyses were conducted in duplicates and the result was presented in an average of the duplicate measurements.

3. Results and Discussion

Our previous study showed that C. striata meat extract was active for ACE inhibition. Using 5 mg extract concentration, the extract was comparable with commercial hypertension drug, captopril (1–5 mg) in inhibiting ACE activity in vitro [3]. In this study, we used a higher concentration of the extract, i.e. 10 mg·mL⁻¹. Among tissue part of C. striata, egg extract showed the highest activity both for ACE inhibition and antioxidant (figure 1). In concentration of 10 mg, egg/gonad of C. striata showed comparable activity in inhibiting ACE as the captopril, followed by meat and gill extract and skin extract. Figure 2 showed the scavenging DPPH activity by different parts of C. striata extracts. Egg/gonad of C. striata also showed the best capability in scavenging DPPH, followed by meat and other parts.

The egg is dedicated to a new generation, therefore, the egg is composed of high-quality material and best environment condition for the egg to be grown. In the early incubation of the chicken egg, the chick embryo is exposed to considerably high oxygen tensions. Therefore, natural defense system in the form of antioxidant material effectively will develop and increase with increasing incubation time [16]. From this, it can be understood that C. striata egg contains the highest antioxidant naturally dedicated for optimal protection of the fish egg. Following egg, meat extract possesses the second highest antioxidant, and this might be due to the high nutritional quality of the C. striata as mention before. A study conducted by previous researchers [15], showed that water-soluble extracts of farmed sea bream meat (Sparus aurata) exhibited better DPPH scavenging activity of radical compared to the wild meat. From this result, it can be inferred that the water extract of fish such as sea bream and C.
*C. striata* especially in the egg and meat contain substance/material that was able to donate hydrogen and acted as electron donors and could react with free radicals to convert them to more stable products.

Cooking is intended primarily for obtaining healthy nutritious food. Fish meat becomes edible and more digestible when they are subjected to cooking. However, loss of the nutritional value of fish due to inappropriate heating will cause undesirable changes in some components of the protein and lipid. For snakehead, grilling was the best cooking for healthy eating of fish since the proximate and mineral were not affected [21]. Different fish such anchovy (*Engraulis encrasicholus*) was recommended also to be cooked by grilling and baking to obtain healthy consumption of anchovy [22].

![Figure 1. ACE inhibition by different *C. striata* tissue extract.](image1)

![Figure 2. DPPH inhibition of different *C. striata* tissue extract.](image2)

In this study, cooking was proved to decrease biological activity of the extract against ACE inhibition. Heat processing methods used were direct boiling (in water), indirect boiling (using a water bath) and steaming, all at 100 °C for 10 min. Direct heat treatment such as steaming is practically applied when people cook snakehead for patients. Both boiling and steaming is a moist-heat cooking
method; boiling uses convection to transfer heat from a hot liquid to the fish meat submerged in it; while steaming is transferred from steam to the fish meat being cooked by direct contact. When uncooked fillet accounted or 100 %, steam and direct boiling reduced the ACE inhibitor activity by about 23 % while indirect heat-treated meat lost 6 %. Accordingly, cooking treatment also affects antioxidant activity, being the detrimental treatment were steaming and direct boiling. Steaming and direct boiling reduces scavenging capability by 37 % (figure 3 and figure 4). Indirect boiling that used a water bath, i.e. the meat was in the beaker which was set having a water temperature of 100 °C, was the least affected. This might be related to the protein coagulation caused by heat treatment, where indirect heat-treated meat has undergone the least coagulation. Consequently, the water-soluble protein content of indirect heat-treated meat was the highest (table 1). A study conducted by previous researchers [15], reported the effect of the cooking method on the antioxidant capacity of water-soluble extract of sea bream fish. The oven-cooked extract had the best scavenging activity on DPPH and hydroxyl radicals of sea bream fish compared to those by boiling and grilling method. From these studies mentioned above, cooking by indirect heating methods and dry-heat cooking showed a better result, retaining both biological activities of the extract and nutritional value of the meat. It can be inferred that the bioactive such as ACE inhibitor and scavenging DPPH activity was in the water-soluble extract, and it may be protein or peptide.

![Figure 3](image.png)

**Figure 3.** ACE inhibition of *C. striata* meat extract (10 mg) treated with different cooking methods.
Figure 4. Effect of cooking methods on the ability of DPPH radical scavenging activity.

Table 1. The protein content of *C. striata* meat soluble fraction after heat treatment.

| No  | Heat treated fillet            | Protein (%) ± SD |
|-----|--------------------------------|------------------|
| 1   | Boiling                        | 4.809 ± 0.2439   |
| 2   | Steaming                       | 3.2349 ± 0.6950  |
| 3   | Indirect heating (water bath)  | 7.6296 ± 0.6278  |

4. Conclusion

*C. striata* egg demonstrated the best activity of ACE inhibition and antioxidant activity followed by meat extract, while gill and skin part had lower activity. *C. striata* egg possessed comparable ACE inhibition activity with captopril in the same application concentration (10 mg·mL⁻¹). Cooking was proved to decrease biological activity of the extract against ACE inhibition and scavenging DPPH activity. Steaming and direct boiling was not a good cooking method for *C. striata* as indirect boiling. Water extract of *C. striata* egg and meat might contain a source of antioxidant and ACE inhibitor substances that is potential for preventing hypertension and another related disease.

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