Antioxidant Capacity of Snakehead Fish Extract (Channa striata) at Different Shelf Life and Temperatures

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Abstract. The albumin-rich of snakehead fish (Channa striata) extract (SHFE), has been developed as medicinal-food due to its antioxidant potential. This investigation was aimed to determining the effect of shelf life (1, 4 and 8 weeks) and temperatures (4°C; 30°C; 50°C) toward SHFE antioxidant capacity using tetramethoxy azobismethylene quinone (TMAMQ) enzymatic-based. The ascorbic acid was used as an antioxidant control. The fluctuated antioxidant capacities were found during different shelf life i.e. at temperature storage of. 4°C (4.17 - 6.49 µM); 30°C (7.72 - 5.58 µM) and 50°C (6.60 – 6.11). Antioxidant capacity of SHFE was approximately 5.7 times higher than ascorbic acid which can to be a frame of reference for further in-vivo/in-vitro research of SHFE and its applications as a pharmaceutical food.

1. Introduction
A free radical is a highly reactive molecular species that can attack important macromolecules in the body such as lipids and proteins, leading to cell damage and homeostatic disruption. [1,2,3] The free radical can be neutralized by the antioxidant molecule that stable enough to donate an electron, hence delay or inhibit cellular damage.4 Antioxidants can be produced in the body as well as from food intake.[5,6,7] Since the snakehead fish extract (SHFE) was reported containing potential-antioxidant compounds, primarily albumin,[8,9,10] it has been developed as medicinal-food.

The preliminary study indicated that SHFE decreasing the MDA (malondealdehyde) concentration and inhibited the pancreatic damage in diabetic rats.[11,12,13] Due to its biopharmaceutical prospective, it is important to conduct the further investigation of antioxidant consistency in SHFE. This investigation was aimed to determining the effect of shelf life (1, 4 and 8 weeks) and temperatures (4°C; 30°C; 50°C) toward SHFE antioxidant capacity using tetramethoxy azobismethylene quinone (TMAMQ) enzymatic-based.

2. Experimental Details
2.1. Preparation of snakehead fish extract (SHFE).
The steam extraction method was performed according to several previous extraction methods8,14,15,16,17 with modification. The unnecessary part of snakehead fish body included fins, scale, gill and digestive organs were removed, while the remaining tissues primarily consist of flesh were rinsed in running water, then it cut into smaller pieces and stored in icy container prior to steam
extraction. The process of steaming was conducted in sterile room using modified steam extractor with temperature and time control equipment. Steaming extractor was adjusted at 50±10°C for 30 minutes. The extract was collected into the sterilized flasks were capped and brought to antioxidant analysis.

2.2. Preparation of Tetramethoxy azobismethylene quinone (TMAMQ) Reagent
TMAMQ was prepared as developed by Prasetyo et al. [18] with modifications. The stock solutions of TMAMQ were produced by incubating the syringaldazine (3.19 mM) within 20 ml of acetone then it were warmed until homogeneous. Citrate buffer (50 mM) at pH 4.5 and 20 ml of laccase were added into solutions of TMAMQ then incubated at for 12 hours at 30 °C in 140 rpm of theromixer (Eppendorf AG, Germany). Furthermore, the solutions were immediately filtered using buchner funnel vacuum filtration and whatmann paper no.1. The powder of filtered TMAMQ onto filter paper then was rinsed with 2 liters of aquadest and incubated in the incubator at 37 °C for 5 hours. The dried filter paper then was stretched out and placed into the microtube prior to store in 4°C. The ethanol then was added in TMAMQ (50% v/v) for stabilized the TMAMQ. The calibration curve of TMAMQ was made according to Prasetyo et al.[18,19] using a Hitachi U-2001 UV-vis spectrophotometer in a single-use cuvette at 530 nm.

2.3. Determining the effect of shelf life and temperatures toward SHFE antioxidant capacity
SHFE in triplicate of sterilized bottles were stored at temperature of 4°C; 30°C (room temperature); 50°C during 1 week, 4 weeks and 8 weeks. SHFE antioxidant capacity were then analyzed using TMAMQ. A fraction (100 μl) of each sample of SHFE was added into solution of TMAMQ (700 μl) then centrifuged at 3000 rpm for 60 seconds. The antioxidant activity of SHFE samples were measured based on the potential of laccase-generated TMAMQ that developed by Prasetyo et al. [18,19] The oxidation activity was observed at 530 nm using a Hitachi U-2001 UV-vis spectrophotometer in a single-use cuvette. Antioxidant capacity of ascorbic acid was analysed as a control. The quantitative data were analyzed descriptively, and qualitative data were analyzed by theoretical approach.

3. Results and Discussion
The change color intensity of the TMAMQ reagents after added with SHFE (from purple became clear) demonstrates at figure 1. The change color intensity of TMAMQ reagents after the addition of SHFE (antioxidants) caused by the withdrawal of free radicals. TMAMQ is a quinone compound formed from the syringaldazine oxidation process by laccase. TMAMQ is stable and have purple color during at pH range of 4.0-7.5 [18]. Antioxidants in SHFE will donate electrons to TMAMQ, thus reducing TMAMQ to syringaldazine.[20] It can change the color becomes clear. The formed syringaldazine will not re-oxidize to TMAMQ because there is no laccase, then the color of the solution will remain clear.

![Figure 1](image-url)  
*Figure 1.* TMAMQ reagents that added with SHFE solution at concentrations sequentially from left to right 0.1; 0.25; 0.5; 0.769 and 1 mg/ml showed decreasing intensity becomes clear
The more concentrated of SHFE showed more significant color disappearance of TMAMQ reagents, which indicates the higher concentration of antioxidants contained therein. SHFE contains antioxidant-potential materials such as albumin, glutathione, Zn minerals as antioxidants as well as glutamic amino acids, cysteine and glycine as precursors of glutathione [8]. Albumin is the most widely contained protein in SHFE extract, which is 2.17 g per 100 ml of extract [8,19,21]. Albumin contains many sulfhydryl (-SH) groups that function as free radical binders [21], while glutathione has been known prevent the accumulation of free radicals thus reducing oxidative stress [22,23,24]. The influence of temperature and shelf life on SHFE antioxidant capacity is presented in Table 1.

Table 1. The Average Of Antioxidant Capacity In SHFE (1 mg/ml).

| Temperature | Shelf life (weeks) | Concentrations of the reduced TMAMQ (µM) |
|-------------|-------------------|----------------------------------------|
| 4°C         |                   |                                        |
| 1           | 4.17              |                                        |
| 4           | 5.66              |                                        |
| 8           | 6.09              |                                        |
| Total 8 weeks | 15.92             |                                        |
| Weekly average | 5.31              |                                        |
| 30°C        |                   |                                        |
| 1           | 7.72              |                                        |
| 4           | 5.16              |                                        |
| 8           | 5.58              |                                        |
| Total 8 weeks | 18.46              |                                        |
| Weekly average | 6.15              |                                        |
| 50°C        |                   |                                        |
| 1           | 6.60              |                                        |
| 4           | 5.47              |                                        |
| 8           | 6.11              |                                        |
| Total 8 weeks | 18.18              |                                        |
| Weekly average | 6.06              |                                        |

Table 1 showed that fluctuated antioxidant capacities were found during different shelf life i.e. at temperature storage of 4°C (4.17-6.49 µM); 30°C (7.72 -5.58 µM) and 50°C (6.60 – 6.11). The highest antioxidant capacity (7.72 µM) was found during shelf life of 1 week at room temperature (30°C).

The lowest antioxidant capacity for total 8 weeks and weekly average was found at 4°C (15.92 and 5.31 µM) is due to the low activity of glutathione peroxidase in SHFE, where the enzyme has an optimal temperature at 30-40 °C [25]. The antioxidant capacity for total 8 weeks and weekly average at 50°C (18.18 and 6.06 µM) was lower than the total 8 weeks and average antioxidant capacity at 30 °C (18.46 and 6.15 µM). This may be caused by inactive of antioxidant proteins in SHFE due to the structural change. During 50°C, the antioxidant proteins in SHFE were possibly broken down by protease enzyme activity which have an optimum temperature of 45-60 ° C [26]. The shelf life also can affect the SHFE's antioxidant content because in a certain time, protease enzymes will break down proteins into simpler molecules, depending on temperature and other factors such as pH [27].

The profile of TMAMQ reduction rate by 1 mg / ml SHFE is presented in Figure 2. This profile showed the similarity trend with reduction rate of TMAMQ by glutathione which previously studied by Prasetyo et al. [18]. The profile of TMAMQ reduction rate by SHFE profile showed similarity with reduction rate of TMAMQ by glutathione which previously studied by Prasetyo et al. [18;19]
The TMAMQ reduction rate profile by glutathione is slower when compared to the TMAMQ reduction profile by ascorbic acid (vitamin C) and α-tocopherol (vitamin E). This is due to the oxidation-reduction potential of the alkenyl thiosulfinate group. The rate of electron donors by the antioxidants in TMAMQ, also affects the reduction rate of TMAMQ to syringaldazine. The comparison of antioxidant capacity between SHFE and ascorbic acid at room temperature without shelf life treatment were measured at level of 10.60 and 1.86 μM respectively. This indicated that SHFE have significant higher antioxidant capacity (approximately 5.7 times) than ascorbic acid. This finding is very important as a frame of reference for further in-vivo / in-vitro research of SHFE and its applications as a pharmaceutical food.

4. Conclusion
The antioxidant capacities of SHFE were fluctuated during different shelf life i.e. at temperature storage of 4°C (4.17-6.49 μM); 30°C (7.72 -5.58 μM) and 50°C (6.60 – 6.11). The highest antioxidant capacity (7.72 μM) was found during shelf life of 1 week at room temperature (30°C). SHFE have significant higher antioxidant capacity (approximately 5.7 times) than ascorbic acid.

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