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Effect of Time of Low-Frequency Ultrasound Treatment on Antioxidant Activity and Physicochemical Properties of Silver Carp Myofibrillar Protein

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***Abstract* – The aim of this research was to study the effect of sonication at a different time on the properties of silver carp myofibrillar protein. The samples were treated by using the ultrasonic technique for 6, 8, 10, 12 and 14 min at 350W. Sonication at different times had a significant effect on antioxidant activity where the DPPH inhibition of samples ranged from 16.07% to 36.51%, and the ABTS inhibition of samples ranged from 14.17% to 22.58%. The treatments by sonication for 12 min showed the highest antioxidant activity, while sonication treatments at different times did not achieve a significant effect on the solubility of samples. The results showed a significant decrease in turbidity when treated with sonication. Water holding capacity significantly increased when the sonication time increased to 12 min. It is clear from the results of this study that the sonication time has an effect on the properties of silver carp myofibrillar protein. Moreover, can be used the sonication treatment to improve the properties of silver carp myofibrillar protein.**

***Keywords* – Silver Carp, Myofibrillar Protein, Low Frequency Ultrasonication, Ultrasound Time, Antioxidant Activity, Physicochemical Properties.**

I. INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*) is one of the main and lowest-costing freshwater fish species harvested commercially in China due to its fast growth rate, easy cultivation, high feed efficiency ratio, as well as high nutritional value [1]. The farmed silver fish muscle content of moisture, crude protein and lipids are 74.03, 20.00 and 2.52%, respectively [2]. Silver carp is freshwater farmed fish that is comparable to the marine fish sources of n-3 PUFA, namely 36% of total fatty acids [3]. Its fillets present an attractive white color, but the unpleasant earthy, musty and fishy odor has restricted the fish species from being accepted by consumers widely [1]. In recent years, there are products which develop from silver carp, such as fermented sausages [4], surimi [5-7], and restructured fish [1].

With the development of the modern food industry, one of the constant challenges is looking for innovative technologies to enhance processing efficiency, and to reduce energy consumption. Concerning this, ultrasound as an innovative green technology has been widely studied in recent years [8].

Ultrasound is a mechanical vibration at a frequency higher than the frequency range, which is audible to the human ear. Ultrasonic energy can be transmitted in solids, liquids, or gases. The use of ultrasound in food

processing can be grouped into two categories: high power-low frequency (up to 10 kW and frequency range of 20-100 kHz) and low power-high frequency (up to 10 W and a frequency of up to 10 MHz) [9]. A number of researchers reported the effect of high intensity-low frequency ultrasound on improving the properties of meat protein due to the modification of proteins. Saleem and Ahmad [10] reported the improvement of gelling properties, regular three-dimensional networks and Water Holding Capacity (WHC) of chicken actomyosin as the influence of ultrasonication at low frequency of 20 kHz; Ru, Liu [11] found the improvement of the solubility and dispersion of silver carp myosin by ultrasound 20 kHz, 100-250 W, 3-12 min; Higuera-Barraza, Torres-Arreola [12] showed the improvement of the emulsifying properties of squid (*Dosidicus gigas)* mantle proteins by pulsed ultrasound (20 kHz, 20 and 40% amplitude, applied for 30, 60 and 90s); and Amiri, Sharifian [13] reported that high-intensity ultrasound (20 kHz, 100 and 300 W, for 10, 20 and 30 min) could enhance pH, reduce particle size, enhanced solubility, WHC, emulsifying, foaming properties and gel strength of beef myofibrillar proteins.

However, there are ultrasound parameters that should be considered in research studies in order to get the best result of the treatment. The type of ultrasound equipment, namely ultrasonic a probe system or ultrasonic cleaning bath; the ultrasonic frequency, usually around 20 kHz for high-intensity ultrasound applications [14]; and determination of the best time and power of sonication. It must be noted that the power and time of ultrasound influence the functional and physicochemical properties of myofibrillar proteins. In some studies, long exposure to ultrasound had damaging effects on the characteristics. Accordingly, the adjust the time of sonication is important for achieving the best results [13]. To the best of our knowledge, the effect of ultrasound treatments on the properties of myofibrillar protein derived from the silver carp has not been previously reported. Therefore, this study aimed to obtain the optimum sonication time and to evaluate the effect of low-frequency ultrasound treatment (20 kHz, 350 W) on physicochemical characteristics and antioxidant activity of silver carp myofibrillar protein.

II. Materials and Methods

2.1. *Raw Material and Chemicals*

Fresh live silver carp fishes (weight = 2-3 kg and length = 51-58 cm) were purchased from a local supermarket (Wuxi, Jiangsu, China). Trained store personnel sacrificed fishes using percussive stunning, then fishes were scaled, eviscerated, decapitated and washed in running tap water. Thereafter, they were covered with crushed ice and kept in icebox, then transported to the laboratory within 15 min. The fishes were skinned and filleted manually. Only white muscles from dorsal muscle were used and the red muscles were manually removed with a knife. Then, fish fillets were placed in polyethylene bags and kept at -60°C prior to the preparation of myofibrils.

Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2-Azinobis (3-ehtylbenzothiazoli- 6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co. (Shanghai, China). Other chemicals and reagents were produced by Sinopharm Chemical Reagent, Shanghai, China. All the chemicals and reagents were of analytical grade.

2.2. *Preparation of Myofibrils Protein*

Myofibrils were prepared according to the method of Qiu, Xia [15] with modifications. Frozen fillets were thawed overnight at 4°C then were cut into small pieces. Two grams of trimmed meats were added with 15 ml

phosphate buffer (4°C, 50 mM, pH 7.5) then homogenized for 30 s (T 10 basic Ultra Turrax, IKA, Staufen, Germany). The homogenates were centrifuged at 10,000 rpm, 4°C for 15 min (Sigma Laboratory Centrifuges 4K15, Germany). The supernatants were decanted and the precipitates were washed again using the same method once more. After that, the precipitates were added with 20 mL phosphate buffer (4°C, 50 mM, pH 7.5) containing 0.6 M NaCl followed by homogenization for 30 s using homogenizer. The homogenates were centrifuged at 10,000 rpm, 4 °C for 15 min. The precipitates were washed again using the same method once more. The obtained supernatants from two times the washing process were collected and mixed manually. The supernatants were filtered through three layer-cloth and used as myofibrillar proteins (MP). Protein concentration was determined using the biuret method Gornall, Bardawill [16] with bovine serum albumin (BSA) as standard. This MP solution was stored at 4°C and was used within 48 h.

2.3. *Sonication Treatment*

Ultrasound treatment of the samples was carried out using a probe system (JY88-II Ultrasonic Cell Disruptor, 500 W, 20 kHz, Scientz Biotechnology Co. Ltd., Ningbo, China) equipped with a horn of 6 mm diameter. The ultrasound intensity was determined calorimetrically using the method of Jambrak, Lelas [17]. The ultrasound intensity was 58.59 W.cm-2 for the power output of 350 W.

The MP solution were adjusted to 3 mg/ml protein concentration with phosphate buffer (4°C, 50 mM, pH 7.5) containing 0.6 M NaCl. Twenty ml of MP solution in 50 ml glass beaker was placed into an ice bath to maintain the solution temperature below 12 °C. Sonication treatments for samples were carried out at 350 W for 0, 6, 8, 10, 12 and 14 min (pulse duration of 4 s to turn on and 1 s to turn off). During ultrasound treatment, the probe was immersed in the MP solution to a depth of 1 cm from the bottom (half depth of the solution). Sample without sonication (0 min) was served as the control. All the samples were stored at 4°C prior to analysis.

2.4. *Antioxidants Activities Assays*

2.4.1. *DPPH Radical-Scavenging Activity*

The DPPH radical-scavenging activity of the samples was determined using the method described by Noman, Qixing [18]. Sample solutions (0.5 mL) were mixed with 3 mL of 0.1 mM DPPH in methanol. The mixtures were shaken by vortex for 10 s and kept for 30 min at room temperature in the dark, and the reduction of DPPH radical was estimated at 517 nm using a UV spectrophotometer (UV-1800PC, Mapada). DPPH scavenging activity was calculated with the following equation:

$$\text{DPPH Inhibition \%} = \frac{\text{Absorbance of the blank} - \text{Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

2.4.2. *ABTS Radical-Scavenging Activity*

ABTS radical scavenging activity of samples was estimated by the method described by [19] with minor modifications. Briefly, 2.6 mM potassium persulfate 7.4 mM ABTS solution was mixed to make a stock solution of ABTS radicals. Equal amounts of stock solutions were mixed to prepare the working solution and allowed to react for 16 h in the dark at room temperature, then diluted by methanol (98%) to get absorption of 0.70 ± 0.02 at 734 nm. 20 µL sample was combined with 3.5 mL of ABTS·+ solution then the mixture was kept at room temperature for 30 min in the dark, and absorbance was measured at 734 nm using a UV spectrophotometer (UV-

1800PC, Mapada). Distilled water was used instead of the sample in the blank sample. The ABTS· + scavenging activity was calculated according to the following equation:

$$\text{ABTS Inhibition}(\%) = \frac{\text{Absorbance of the blank} - \text{Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

2.5. *Protein Solubility Measurement*

The solubility of MP solutions was measured according to the method of Anon, De Lamballerie [20] with modifications. An aliquot of 4 mL MP solutions (3 mg mL-1) was centrifuged (Sigma Laboratory Centrifuges 4K15, Germany) at 10,000 rpm, 4°C for 20 min. The protein content in the obtained supernatant was measured using the Biuret method [16]. Protein solubility was expressed as a percentage of supernatant protein concentration and protein concentration before centrifugation. Three replicates were performed for each treatment group.

2.6. *Turbidity Determination*

The turbidity of the MP solution was determined using a modified version of the procedure reported earlier [21]. The absorbance of samples at 660 nm was measured using UV-VIS Spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) to reflect the turbidity values.

2.7. *WHC Measurement*

WHC of MP gels was measured with some modifications of the centrifugation technique described by Zhang, Regenstein [22]. Two mL of MP solutions were transferred to 4 mL capped plastic centrifuge tubes. The tubes were heated in a water bath at 40°C for 30 min, and at 90°C for 20 min. The protein gels were then immediately cooled in ice-cold water for 1 h and were stored at 4°C for 20 h. The tubes were centrifuged at 10,000rpm for 20 min (TGL-16C, Anke Instrument Ltd. Co., Shanghai, China). The supernatants were decanted then the residual water was carefully discarded using Whatman filter paper. WHC was calculated by the following equation:

$$\text{WHC} = \frac{\text{Protein gel (g)} - \text{Protein content(g)}}{\text{Protein content(g)}}$$

2.8. *Statistical Analysis*

All data were expressed as means ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to determine the statistical difference. Significant differences between means were identified using Duncan's multiple range test ($p < 0.05$). Statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM SPSS software, USA).

III. Results and Discussion

3.1. *Antioxidant Activities*

3.1.1. *DPPH Radical-Scavenging Activity*

The scavenging capacity of myofibrillar protein was measured versus DPPH, considered a stable free radical with a maximum absorbance at 517 nm. Ktari, Jridi [23] reported that when DPPH faced a hydrogen-contributing substance (H+), the radical was scavenged and the color changed from purple to yellow, which caused a decrease of absorbance. The antioxidant activity of MP samples against DPPH was shown in Fig. 1. The DPPH inhibition

of samples ranged from 16.07% to 36.51%. It is clear from the results that the treatment of samples by ultrasound at a different time has a significant effect on their antioxidant activity, where the highest antioxidant activity was achieved when the sample was treated by ultrasonic for 12 min, which was 36.51%. The radical scavenging activities of proteins are usually related to the amino acid profile in terms of the difference in the sequence, amino acid composition, size of the peptide, and hydrophobic property resulting from different treatments [24]. On the other hand, Phongthai, Lim [25] reported that the essential amino acids such as histidine, methionine, phenylalanine, and tyrosine, which have been found to be stronger proton donors from other amino acids. This result was supported by the previous study [26]. The antioxidant activity of porcine liver protein hydrolysates (PLPHs) as ultrasonication time increased to a certain duration (60 s). The hydrolysate pretreated with ultrasonication (40 kHz, 400 W) for 30 s exhibited the highest (DPPH) radical scavenging activity. The ultrasonic pretreatment assists structures unfolding of protein as well as the destruction of hydrophobic interactions of protein molecules.

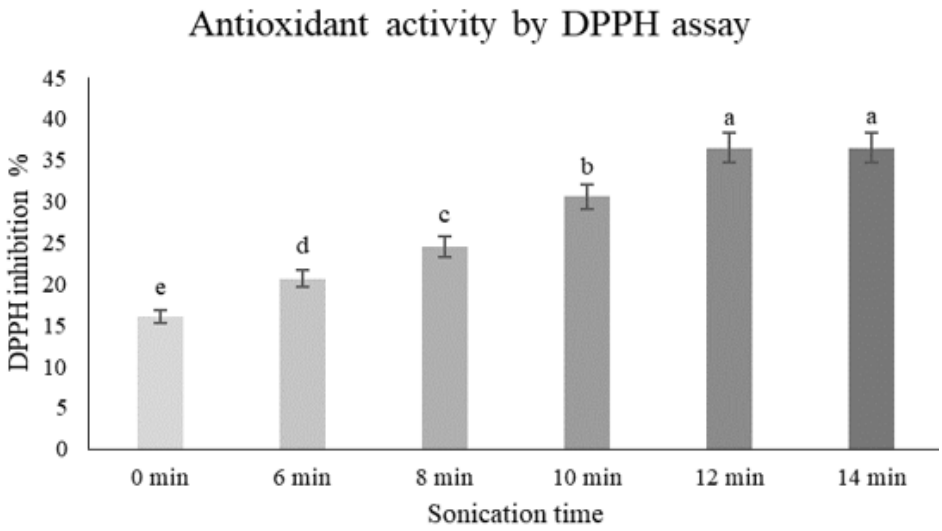

Fig. 1. Antioxidant activity of the myofibrillar protein of Silver carp fish by DPPH assay.

3.1.2. *ABTS Radical-Scavenging Activity*

ABTS assay is an important method for estimating the antioxidant activities of hydrogen-donating materials, where ABTS radical is stable and can be reduced by antioxidants compounds [27]. The antioxidant activity of the samples against ABTS was estimated and the results are presented in Fig 2. Ultrasonic treatments at different times resulted in significant changes in the antioxidant activity of the samples. The ABTS inhibition of samples ranged from 14.17% to 22.58%. The best antioxidant activity is shown in the sample which had treatment by ultrasound for 12 min. The differences in radical scavenging activity between different treatments may be due to changes in their composition of amino acids and the molecular weight of peptides resulting during treatments [28]. The result was in line with the previous report by Zou, Yang [29]. Sonication treatment (20 kHz, 200 W for 20 min) increased significantly the antioxidant activity (ABTS radical scavenging activity) of chicken plasma protein. This may be ascribed to an enhancement of hydrophobic proteins or amino acids materials after sonication. However, the additional sonication (30 min) showed a decline of antioxidant activity which may cause by over processing in the tertiary structure, particle size, surface hydrophobicity, and extensive protein denaturation.

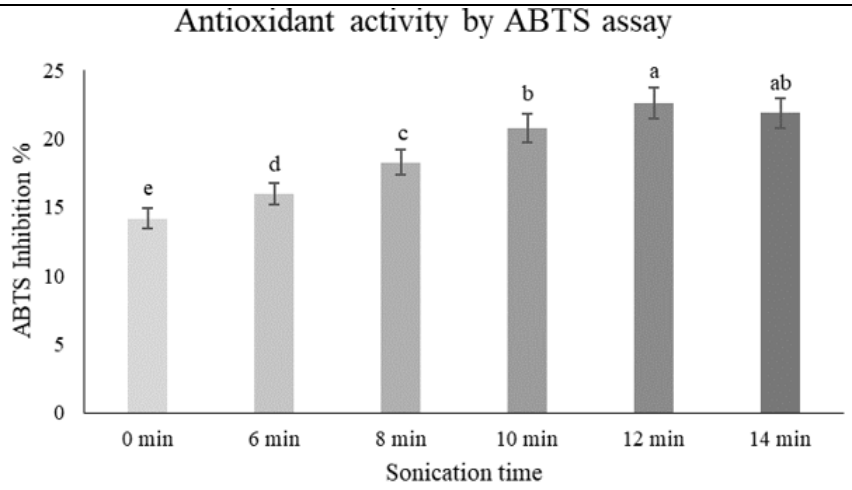

Fig. 2. Antioxidant activity of the myofibrillar protein of Silver carp fish by ABTS assay.

3.2. *Solubility of Myofibrillar Protein*

Protein solubility is an important and useful functional property for different food applications, where the solubility affects other functional characteristics of proteins like emulsifying and foaming [30]. The results of the solubility of myofibrillar protein are displayed in Fig. 3. The solubility of Silver carp myofibrillar protein ranged between 85.63 and 89.79% with non-significant different under effect the ultrasonic treatment time. These results are considered high and may be attributed to protein unfolding during the ultrasonic treatment that leads to polar amino acids being exposed and an increase in protein solubility [31]. Beside it, ultrasound treatment could reduce the particle size of protein which provides more surface area and greater charge. It contributed to stronger protein-water interaction, which intensifies the protein solubility [22].

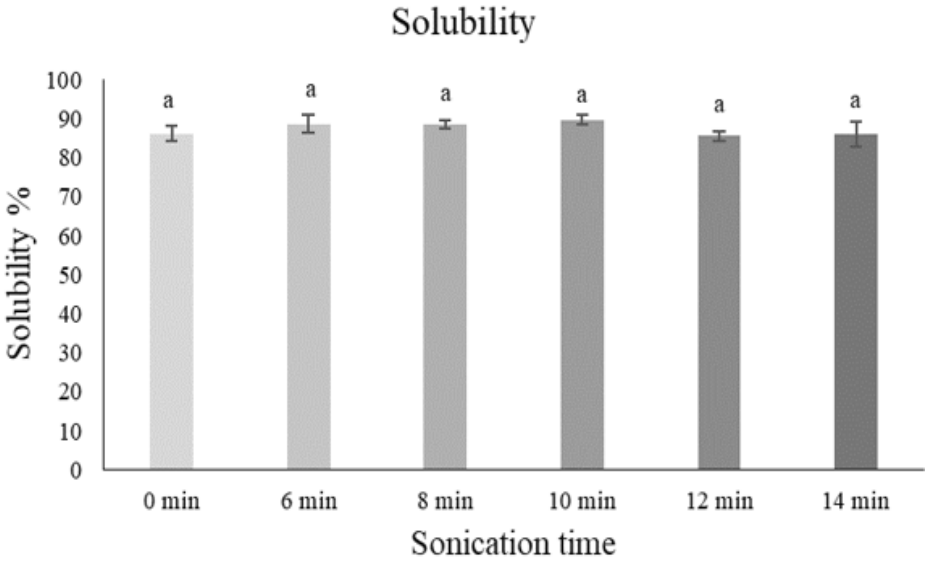

Fig. 3. Solubility of myofibrillar protein of Silver carp fish at various sonication times.

3.3. *Turbidity*

Turbidity of myofibrillar protein of Silver carp fish samples showed in Fig 4. The turbidity of myofibrillar protein samples ranged from 0.056 to 0.251. Statistical analysis showed significant differences ($p \leq 0.05$) between the results in terms of turbidity. The control sample showed the highest turbidity, while the turbidity decreased significantly when the samples treated by sonication. The treatment time had a significant effect on the turbidity

of the samples except at 12 min and 14 min. Benjakul, Visessanguan [21] reported that increased turbidity means increased protein.

The turbidity of protein pellets was correlated to the content of protein aggregate [21]. Zhang, Regenstein [22] reported that ultrasonication treatments could decrease the turbidity of chicken MP solution. This was associated with the smaller particle size due to high shear energy waves and turbulence from the cavitation phenomenon. Sonication was caused particles were agitated violently, broken, decreased in diameter of protein particles furthermore increased in the specific surface area which available for light scattering.

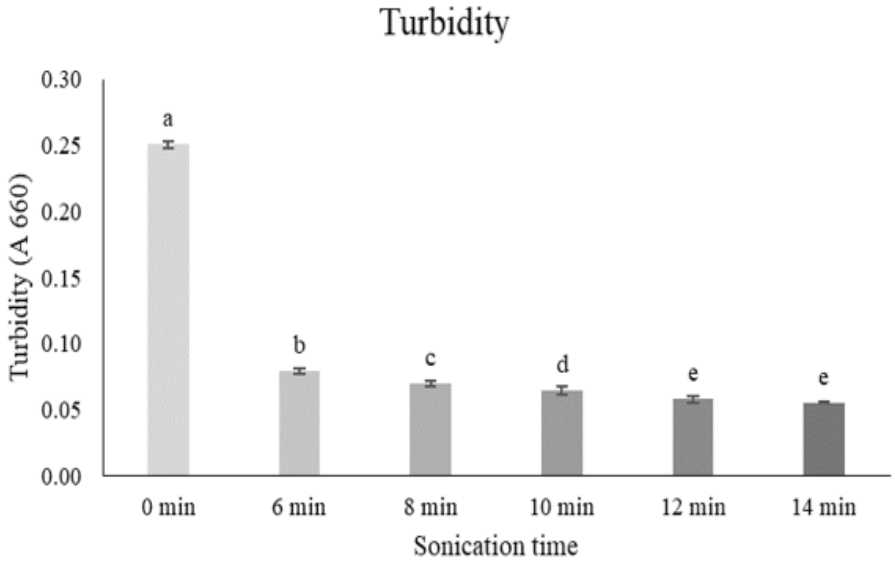

Fig. 4. Turbidity of myofibrillar protein of Silver carp fish.

3.4. *Water Holding Capacity*

The functional properties of proteins in food systems generally depend on water-protein interactions. The ability of the protein to absorb water and retain it against a gravitational force within a protein matrix is known as WHC [32]. Figure 5 shows the WHC of Silver carp myofibrillar protein, where WHC ranged between 1.35 and 5.41 (g Water/g myofibrillar protein) with significant differences in all treatments. The sample treated by sonication for 12 min achieved the highest result, while the sample treated for 6 min showed the lowest WHC. There was a significant difference ($p \leq 0.05$) between all samples in terms of water holding capacity. The polar groups, such as – COOH and – NH2 resulting from treatment may affect water absorption [18].

The previous studies reported that ultrasonic treatments could significantly affect the water holding capacity of variable muscle protein, namely fish [33], chicken [10, 22] and bull [13]. Reducing the particle size of protein due to sonication lead networks of protein gels become denser and uniform which promotes the water binding in the gels. As a result, the homogenous and fine of gel structures have a higher of WHC values compared to nonhomogeneous of gel structures. Small pores of the homogenous structure of protein gels could entrap water molecules firmly [13, 22, 33]. However, the longer sonication time or higher of sonicator power could lead the declining of WHC values. This phenomenon may be caused by the thermal effect which made protein denatured [33] or by the more presence of heterogeneous gels [22].

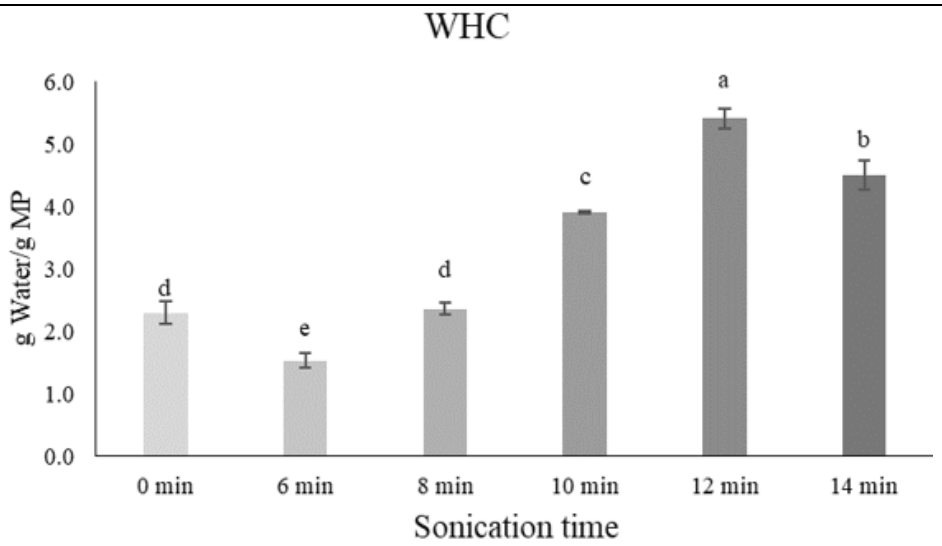

Fig. 5. Water-holding capacity of myofibrillar protein of the Silver carp fish.

IV. Conclusions

In the present study, low-frequency ultrasound treatment significantly affected the properties of the myofibrillar protein of silver carp such as antioxidant activity, turbidity, and water holding capacity. The optimal sonication time is 12 min. The treated sample by ultrasonic for 12 min achieved the best antioxidant activities of DPPH and ABTS. The treatment time of ultrasound did not significantly affect the solubility of myofibrillar protein obtained from silver carp. This study concluded that ultrasound can be used to improve the properties of the silver carp myofibrillar protein, thus add more benefit from this fish species. The results of this study could be helpful particularly in the field of myofibrillar protein. However, other conditions by using ultrasonic should be further studied to prepare myofibrillar protein.

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