International Journal of Agriculture Innovations and Research
Volume 8, Issue 2, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 10/09/2019 | Accepted on: 26/09/2019 | Published: 05/10/2019

Influence of pH-shifting Combined with Low-Frequency Ultrasound on Antioxidant Activity and Physicochemical Properties of Silver Carp Myofibrillar Protein

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Abstract – **This study aimed to investigate the effect of pH-shifting combined with low-frequency ultrasound on antioxidant and physicochemical properties of silver carp myofibrillar protein (MP). The antioxidant activity of the samples against DPPH ranged from 14.31% to 36.43%, and ABTS scavenging between 3.55 and to 22.56%. The ultrasonic treatment of the sample without changing the pH led to the best antioxidant activity against DPPH and ABTS. Protein solubility affected by treatments, which ranged from 30.11 to 88.26%, where the best solubility was at pH 8 with ultrasonic treatment which showed no significant difference to sample control. The turbidity increased in the treated samples at different pH levels without ultrasonic treatment, where the highest turbidity was 0.21 (A600) at pH 6 without ultrasound treatment, while the lowest turbidity was 0.03 (A600) at pH 12 with ultrasound. The highest water holding capacity of MP was achieved at pH 2 with ultrasonic treatment, which amounted to 7.83 g water/g MP. The results of this study indicate that the ultrasonic treatments of the samples at different pH significantly affected the antioxidant activities and physicochemical properties of MP obtained from silver carp.**

Keywords – **Silver Carp, pH-shifting, Myofibrillar Protein, Ultrasonication, Antioxidant Activity, Physicochemical Properties.**

I. INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*) is one of the prime and least expensive species of the freshwater fish commercially harvested in China because of its high nutritional value, rapid growth rate, high nutrition efficiency, as well as easy cultivation (Wang, Zhang, Mujumdar, & Mothibe, 2013). The content of cultured silver fish muscles of fat, crude protein, moisture are 2.52, 20.00 and 74.03%, respectively (Ashraf, Zafar, Rauf, Mehboob, & Qureshi, 2011). Silver carp is freshwater farmed fish rich in n-3 polyunsaturated fatty acids (PUFA) resembling marine fish sources (36% of total fatty acids) (Li, Sinclair, & Li, 2011). The Silver carp slices offer an attractive white color, but the fishy odor and the unpleasant earthy have limited consumers from accepting fish species widely (Wang et al., 2013). In the past years, there are products developed from silver carp, like surimi (Fu et al., 2012; H. Liu, Gao, Ren, & Zhao, 2014), fermented sausages (Xu, Xia, Yang, & Nie, 2010), and restructured fish (Wang et al., 2013).

As the modern food industry develops, one ongoing challenge is looking for novel technologies to improve processing performance and minimize energy loss. In this regard, ultrasound as a novel green technology has been widely investigated in recent years (Xiong et al., 2016).

The mechanical vibration with a frequency higher than the frequency range, which is audible to the human ear is named Ultrasound. Ultrasonic energy can be transmitted in gases, liquids, and solids. The utilize of ultrasound in the food industry can be divided into two categories: low power-high frequency (up to 10 W and a frequency of up to 10 MHz) and high power-low frequency (up to 10 kW and frequency range of 20–100 kHz) (Bekhit, Carne, Ha, & Franks, 2014). A number of investigators have reported the effect of high intensity-low frequency ultrasound on improving meat protein properties due to protein modification, Saleem and Ahmad (2016) Improved sweating properties, three-dimensional regular networks and water holding capacity (WHC) for chicken actomyosin were reported as an ultrasound effect at low frequency 20 kHz; Ru, Liu, Xiong, Fu, and Chen (2017) Improved dispersion and solubility of silver carp myosin by ultrasound 100-250 watts, 20 kHz, 3-12 min; Higuera-Barraza et al. (2017) showed improved emulsification characteristics of squid proteins (*Dosidicus gigas*) by pulsed ultrasound (20 and 40% amplitude, 20 kHz, applied for 30, 60 and 90 seconds); and Amiri, Sharifian, and Soltanizadeh (2018) informed that high-intensity ultrasound (100 and 300 watts, 20 kHz, for 10, 20 and 30 minutes) can improve reduce particle size, pH, increase solubility, foam properties, WHC, gel strength, and emulsification for beef myofibrillar proteins.

The formation of proteins gel is affected by many factors, such as temperature, protein concentration, ionic strength, muscle type, pH, and pressure (X. D. Sun & Holley, 2011; Totosaus, Montejano, Salazar, & Guerrero, 2002). Among these factors, pH is a critical factor which can affect the charge distribution and dissociation state of protein amino acid residues in the solution and can alter the protein intermolecular electrostatic interactions, and thus affect the gel properties and protein molecular structure (Lin & Park, 1998).

One of the most critical factors that influence the gelation of myofibrillar proteins is pH. The optimal pH for gelation is often recorded between 5.5 and 7.0, which depends on the concentration of the protein and the species from which the proteins are obtained (Chang, Feng, & Hultin, 2001). Several studies have shown that elastic gels at a high WHC value could be formed directly by washing chopped muscle with water contained physiological ionic strength (0.15) without need to dissolve the fibrous muscle proteins using high salt concentrations (Feng & Hultin, 2001). Gel formation theories of muscle proteins suggest that the important first step is solubilization of myosin. The gelling ability decreases significantly as the pH decreases from a neutral to a slightly acidic one (e.g., 6.4). Gelation of the insoluble myofibrillar proteins needs neutral or slightly alkaline pH (Yamamoto, Samejima, & Yasui, 1987).

The functional properties of animal and plant proteins could be modified by physical, chemical and biological methods. The traditional and simple method which widely used among chemical treatments is pH-induced modification. In the pH-shifting treatment, the pH of the protein solution is adjusted o an extreme acidic or basis pH value to unfold protein. Then the pH is adjusting back to neutral to refold the protein (Jiang et al., 2017). There are the previous studies on application this method at fish muscle protein, i.e. Sun et al. (2019) who reported the effects of pH shifting on conformation and gelation properties of myosin from blue round scads muscle.

There is insufficient information on the effect of pH-shifting and low-frequency ultrasound combined on properties of silver carp myofibrillar protein. Therefore, the present study aimed to evaluate the effectiveness of pH-shifting and low-frequency ultrasound combined on the antioxidant activities and physicochemical properties of silver carp myofibrillar protein at different levels of pH and ultrasonic treatment.

II. Materials and Methods

2.1. *Raw Material*

Live silver carp fish that weighs between 2 to 3 kg and their length ranges from 51 to 58 cm, was purchased from the local supermarket (Wuxi, Jiangsu, China) and immediately transported to the laboratory on ice. Upon arrival at the laboratory, the sample was washed, eviscerated, removed the skin, and hand filleted. Just the white muscles of the dorsal muscles were used and the red muscles were removed. The fish fillets were then placed in polyethylene bags and kept at -60 °C before myofibrils were prepared.

2.2. *Chemicals*

Bovine serum albumin (BSA) was obtained 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). All other reagents and chemicals were of high purity and analytical grade.

2.3. *Myofibrils Protein Preparation*

Myofibrils protein (MP) was prepared by the recommended method by Qiu, Xia, and Jiang (2013) with some modifications. Frozen fishes fillets were thawed overnight at 4°C, then the fishes fillets were cut into small pieces. Briefly, 15 mL phosphate buffer (4°C, 50 mM, pH 7.5) was added to 2 g of fishes fillets and homogenized for 30 s (T 10 basic Ultra Turrax, IKA, Staufen, Germany), then centrifuged (Sigma Laboratory Centrifuges 4K15, Germany) 10,000 rpm for 15 min at 4°C.

The supernatants material was decanted and the precipitates were washed again using the same method again. Thereafter, 20 mL phosphate buffer (4 °C, 50 mm, pH 7.5) containing 0.6 M NaCl was added to precipitates then homogenization for 30 s using the homogenizer. Furthermore centrifuged at 10,000 rpm, for 15 min at 4°C. The precipitates were washed repeat one time. The collected supernatants from two times washing process were obtained and mixed manually. The supernatant was filtered through three layers of fabric and used as MP. The biuret method was used for determining protein concentration according to Gornall, Bardawill, and David (1949), the bovine serum albumin (BSA) was used as standard. This MP solution was stored at 4°C and was used for tests within 48 h.

2.4. *Myofibrils Treatment by pH-shifting Combined Sonication*

Ultrasound treatment of the samples was performed using a probe system (JY88-II Ultrasonic Cell Disruptor, 500 W, 20 kHz, Scientz Biotechnology Co. Ltd., Ningbo, China) equipped with a 6 mm diameter horn. The intensity of ultrasound was determined calorimetrically according to the method Jambrak, Lelas, Mason, Kresic, and Badanjak (2009). The ultrasound intensity was 58.59 W.cm-2 for the power output of 350 W.

Twenty mL of MP solution (pH 7.3) were diluted with the phosphate buffer (4°C, 50 mM, pH 7.5) containing 0.6 M NaCl to get 3 mg/ml protein concentration, and then adjusted to pH 2, 4, 6, 8, 10 or 12 by using 2 M NaOH or 2 M HCl at room temperature (Jiang et al., 2017). Immediately sonication treatment was applied to the 20 mL protein dispersion in a beaker (50 mL) that was placed in an ice bath to avoid overheating (to maintain the solution temperature below 12°C). Sonication of samples were carried out at 350 W for 12 min (pulse duration of 4 s. on and 1 s. off). During ultrasound treatment, the probe was immersed in the MP solution to depth of 1 cm from the

bottom (half depth of the solution). Treated MP solution was held at room temperature for 1 h before adjusting pH back to 7.3 using 2 M NaOH or 2 M HCl. The supernatant was collected after centrifugation (Sigma Laboratory Centrifuges 4K15, Germany) at 10,000 rpm, 4°C for 15 min and stored in a refrigerator at 4°C before use to analysis. The sample treated with 12 min sonication in combination with pH-shifting was labeled as pH2 + U12, pH4 + U12, pH6 + U12, pH8 + U12, pH10 + U12, or pH12 + U12. The one treated with only pH-shifting or ultrasonic was denoted as pH2, pH4, pH6, pH8, pH10, pH12 and U12, respectively. The sample with no treatment was used as the control. All measurements were taken in triplicate.

2.5. *Antioxidant Activities Determination*

2.5.1. *DPPH Radical-Scavenging Assay*

The radical DPPH activity of the samples was determined according to the method of Noman et al. (2019). The sample solutions (0.5 mL) were mixed with 1.5 mL of 0.1 mM DPPH in methanol. The mixtures were vortexed for 10 s and left for 30 min at 25°C in the dark. Finally, the radical DPPH decrease was determined by UV spectrophotometer (UV-1800PC, Mapada) at 517 nm. The DPPH survey activity was calculated by:

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

2.5.2. *ABTS Radical-Scavenging Assay*

The ABTS radical activity of the samples was performed depending on the method described by Najafian, Jafarzade, Said, and Babji (2013) with some modifications. In short, 2.6 mM potassium persulfate and 7.4mM of ABTS solution were mixed to get a stock solution of ABTS radicals. Then, the working solution was prepared by mixed the equal amounts of stock solutions and allowed to react for 16 hours in the dark at room temperature, after that diluted by methanol (98%) to get absorption of 0.70 ± 0.02 at 734 nm. Thereafter, 20 µL from the sample was mixed with 3.5 mL of ABTS·+ solution. The mixture left in the dark for 30 min at room temperature. Next, the measurement of absorbance was performed by UV spectrophotometer (UV-1800PC, Mapada) at 734 nm. Distilled water was used without sample as the blank sample. The ABTS·+ activity was calculated depending on the following equation:

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

2.6. *Protein Solubility Measurement*

For measurement the solubility of MP solutions, we used the method of Anon, De Lamballerie, and Speroni (2012) with modifications. Four mL MP solutions were centrifuged (Sigma Laboratory Centrifuges 4K15, Germany) 10000 rpm, for 20 min at 4°C. The content of the protein in the obtained supernatant was estimated using the Biuret method (Gornall et al., 1949). Protein solubility (%) was expressed as a percentage of protein concentration in the supernatant and protein concentration before centrifugation.

2.7. *Turbidity Analysis*

MP solution turbidity was determined using a modified method of the previously reported procedure by Benjakul, Visessanguan, Ishizaki and Tanaka (2001). Absorption of samples was measured at 660 nm using a UV-VIS Spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) to reflect the turbidity values.

2.8. *WHC Measurement*

WHC of MP gels was estimated by centrifugation technique described by Zhang, Regenstein, Zhou, and Yang (2017) with some modifications. Four mL of MP solutions was put into 4 mL 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. Then centrifuge the tubes at 10,000 rpm for 20 min (TGL-16C, Anke Instrument Ltd. Co., Shanghai, China). The supernatants material was poured and the remaining water was carefully removed by using Whatman filter paper. WHC was calculated by the following equation:

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

2.9. *Statistical Analysis*

All measurements were done in triplicate. 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 Assay*

The DPPH• radical-scavenging activities of myofibrillar protein obtained under different pH levels and pH/ultrasonic treatments combined were shown in Fig.1. The DPPH inhibition results ranged from 14.31% to 36.43%. From Fig.1, it is clear that the ultrasonic sample at neutral pH achieved the highest result, which was 36.43%. The results showed that the changing pH led to change the antioxidant activity, where the best activity was at pH 8 with ultrasonic treatment, where achieved 28.73%, followed by the treated sample at pH 4 and 6, which were 25.77 and 25.96%, respectively, with no significant difference at $p < 0.05$. Among the samples treated with different pH, the sample at pH 6 achieved the best activity (22.70%) with significantly increased compared to the pH control sample, which was 15.99%. Generally, ultrasonic treatment of the sample without changing the pH led to the best antioxidant activity, therefore it could be concluded that the treatment of samples by ultrasound at neutral pH is better than ultrasound treatment at different pH values. The differences in the result of radical scavenging ability may be attributed to the peptide length and the different amino acid composition of peptides within the protein (Jemil et al., 2014). This result was in line with the previous study by Yu and Tan (2017). The antioxidant activity of porcine liver protein hydrolysates (PLPHs) increased to a certain duration of ultrasonication. This was caused by the ultrasonic pretreatment assists structures unfolding of protein as well as the destruction of hydrophobic interactions of protein molecules.

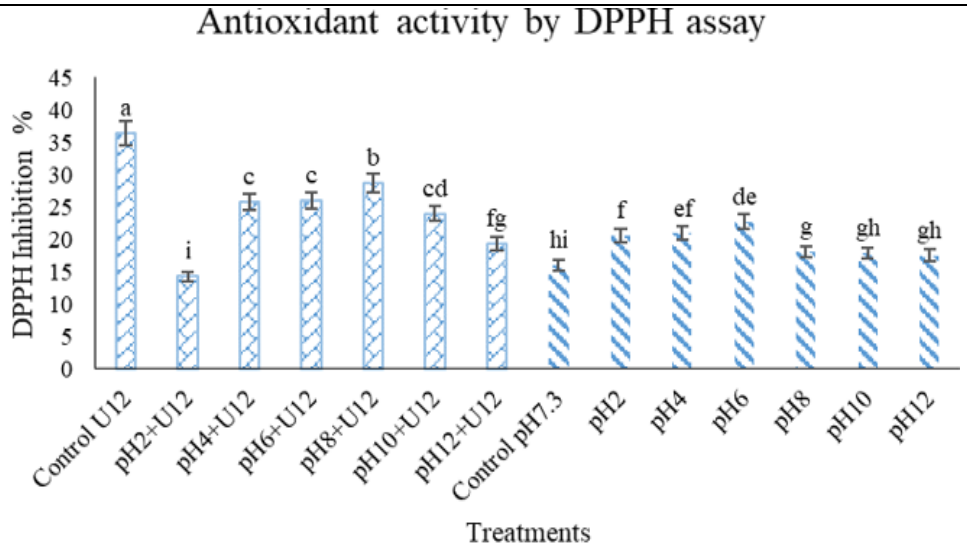

Fig. 1. Antioxidant activity myofibrillar protein of silver carp by DPPH assay.

3.1.2. *ABTS Radical-Scavenging Assay*

ABTS+ radical-scavenging activity assay has been generally used as a measure of the antioxidant activity assay (Najafian et al., 2013). The antioxidant activity of the samples was against ABTS evaluated and the results are shown in Fig 2. The ABTS inhibition results ranged from 3.55 % to 22.56%. Changing the pH of samples, particularly at pH 2, 8, 10, and 12 resulted in the reduction of antioxidant activity compared to the control sample pH 7.3, while samples of pH 4 and 6 showed higher activity than the control sample.

On the other hand, treatment of the samples by the ultrasonic at all pH levels led to decrease the antioxidant activity of the protein samples compared to the control sample of U12.

In general, it can be observed that changing pH reduced antioxidant activity compared to samples treated by ultrasonic without changing the pH.

The result was supported by the previous study (Zou et al., 2018). Sonication treatment (20 kHz, 200 W for 20 min) increased significantly the chicken plasma protein antioxidant activity (ABTS radical scavenging activity). This may be caused by an enhancement of hydrophobic proteins or amino acids materials after sonication.

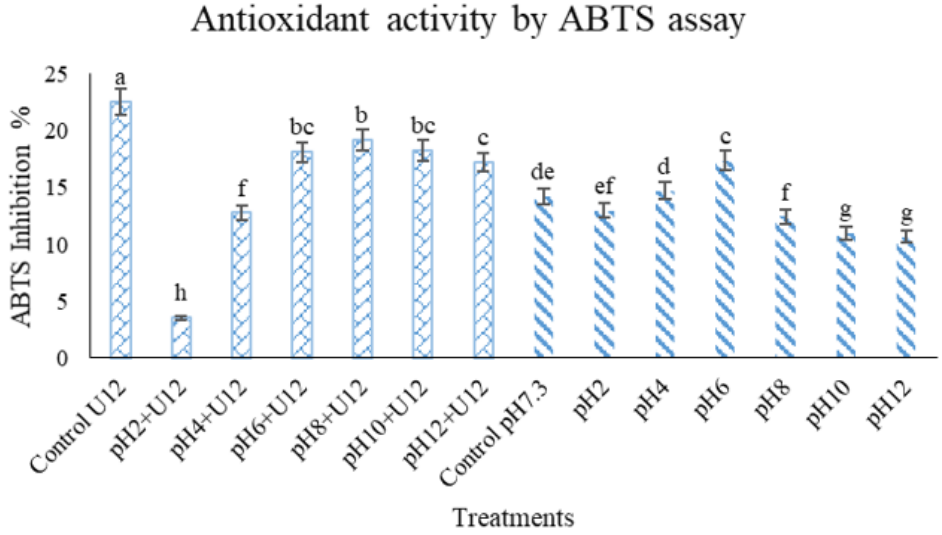

Fig. 2. Antioxidant activity myofibrillar protein of silver carp by ABTS assay.

3.2. *Protein Solubility*

The solubility of myofibrillar silver carp protein was evaluated under the effect of a wide range of pH levels (2-12) and pH/ultrasonic treatment combined as shown in Fig. 3. Changing the pH of the samples generally led to a decrease in the solubility compared to the control sample, particularly the treatment of pH 2, where the solubility of protein decreased to 51.37% from 86.48% in the neutral control sample, while the solubility of other samples decreased but with a lower rate.

On the other hand, using the ultrasonic treatment at different pH levels led to lower solubility especially at pH 2, where the solubility significantly decreased from 88.26% in the neutral control sample to 30.11% the treated sample at pH 2. From the results of Fig. 3, clearly observed that the ultrasonic treatment has achieved better solubility, where pH change led to a negative effect on protein solubility. Jiang et al. (2017) reported the ultrasound treatment gives a more positive effect than pH shifting on solubility of pea protein. Also the protein solution with extremely low pH treatment showed the lowest solubility value. This may be due to impact structure of protein near isoelectric point. Connolly, Piggott, and Fitz Gerald (2014) reported that the ultrasonic treatment leads to polar amino acids being exposed and an increase in protein solubility. In addition, 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 (Zhang et al., 2017).

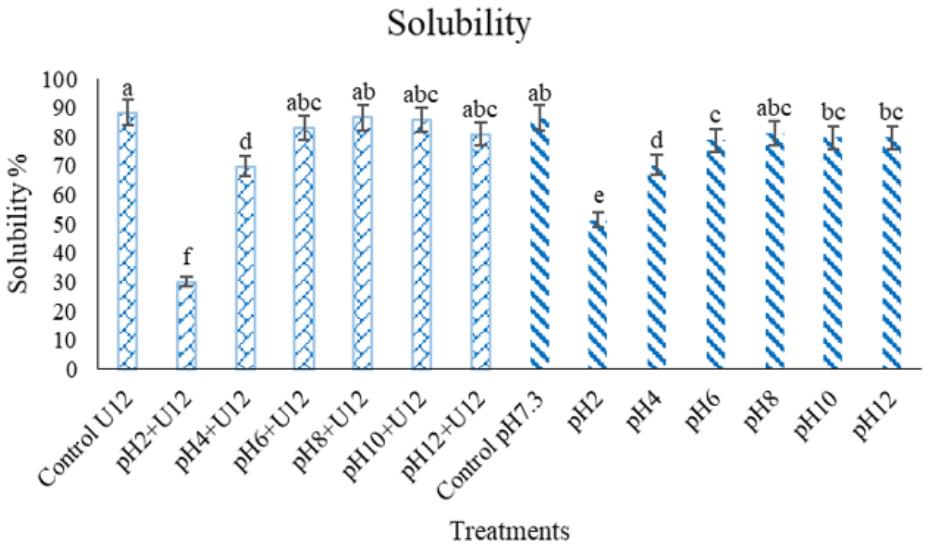

Fig. 3. Solubility myofibrillar protein of silver carp

3.3. *Turbidity*

The turbidity of myofibrillar protein silver carp ranged from 0.03 to 0.21 as shown in Fig. 4. Changing the pH of the samples significantly increased turbidity, especially at pH 4 and 6, where their turbidity was higher than the turbidity of the control sample. In contrast, the ultrasonic treatment led to a significant decrease in turbidity. The lowest turbidity was obtained when the pH of the sample was changed to pH 12 and ultrasonically treated. Benjakul et al. (2001) reported that turbidity change in solution indicates the formation of a total protein during the heating process and absorbance reading commonly used to monitor the extent of protein aggregates. Jiang et al. (2017) showed that the pH-shifting alone treatments increased the pea protein turbidity, which may be caused by the large particle sizes of protein solution. Vice versa, Zhang et al. (2017) concluded 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.

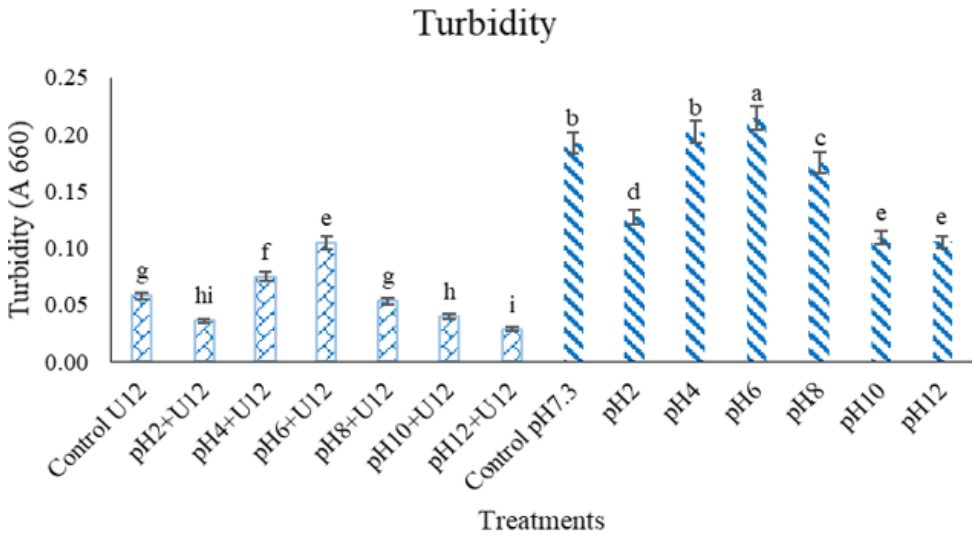

Fig. 4. Turbidity myofibrillar protein of silver carp.

3.4. *Water Holding Capacity*

WHC of myofibrillar protein is presented in Fig. 5. Myofibrillar proteins obtained at pH 2 and 4 with ultrasonic treatment combined achieved the highest WHC values compared to the control sample and other treatments, which were 7.83 and 6.41 g water/g MP, respectively.

On the other hand, different pH levels used in MP preparation led to different WHC values, where the WHC of protein obtained at pH 2, 4, 6 and 8 was higher than the neutral control sample, while the WHC of protein obtained at pH 10 and 12 was lower than the control sample. The high WHC of myofibrillar protein that they could be used as functional ingredients in food formulations to modify texture and viscosity, to reduce dehydration during storage (Jemil et al., 2014).

R. Liu et al. (2010) notified that increasing pH from 5.5 to 8.0 could increase the WHC values of silver carp myosin. Above the pI, myosin swelled and bound a large volume of water due to many charged groups and repulsive forces. As the shifting pH close to pI, proteins tended to coagulate due to increased protein-protein interactions. In addition, protein hydration gradually became weak due to the changes in the hydration states of charged amino acids. So that the WHC of myosin gel decreased with decreasing pH.

Meanwhile, the previous studies reported that ultrasonic treatments could significantly affect the water holding capacity of muscle protein (Amiri et al., 2018; Saleem & Ahmad, 2016). 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 homogenous structure of protein gels could entrap water molecules firmly (Wen, Tu, Zhang, Wang, & Chang, 2017; Zhang et al., 2017). 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 (Wen et al., 2017) or by the more presence of heterogeneous gels (Zhang et al., 2017).

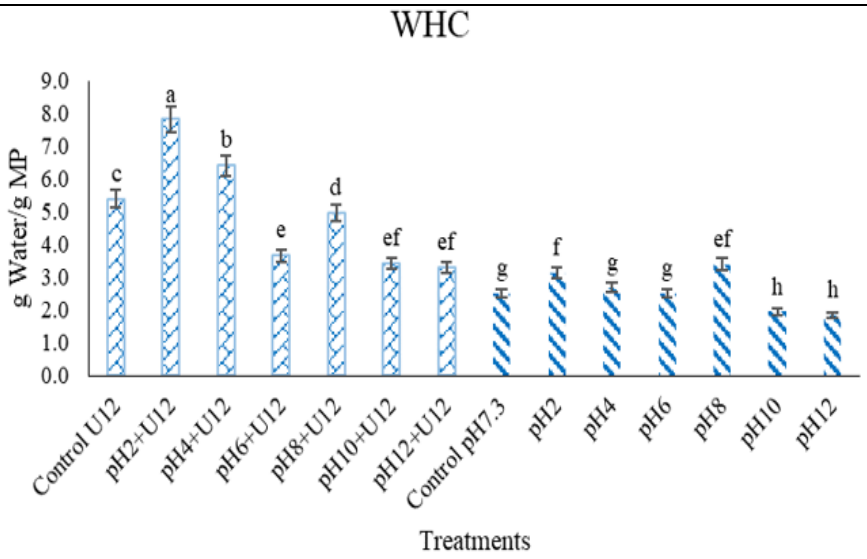

Fig 5. WHC myofibrillar protein of silver carp.

IV. Conclusions

Ultrasonic treatment of silver carp myofibrillar protein at 350 W and 12 min enhanced the antioxidant activities, in addition to improving the protein solubility. The application of ultrasound could reduce turbidity of protein solutions. Moreover, the ultrasonic treatment could increase the water holding capacity, especially at low pH. The exposure of ultrasound on protein solution at different pH levels led to lower solubility particularly at pH 2. Further studies are required to investigate the other modifications of ultrasound treatment conditions to obtain the potential applications of myofibrillar protein.

Acknowledgements

This research was financially supported by China Agriculture Research System (CARS-45-26), Project from Jiangsu Fishery Administration (Y2017-30) and China Scholarship Council (CSC), Beijing, China.

References

[1] Amiri, A., Sharifian, P., & Soltanizadeh, N. (2018). Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins. *International Journal of Biological Macromolecules, 111*, 139-147.
[2] Anon, M. C., De Lamballerie, M., & Speroni, F. (2012). Effect of high pressure on solubility and aggregability of calcium-added soybean proteins. *Innovative food science & emerging technologies, 16*, 155-162.
[3] Ashraf, M., Zafar, A., Rauf, A., Mehboob, S., & Qureshi, N. A. (2011). Nutritional values of wild and cultivated silver carp (Hypophthalmichthys molitrix) and grass carp (Ctenopharyngodon idella). *International Journal of Agriculture and Biology, 13*(2).
[4] Bekhit, A. E.-D. A., Carne, A., Ha, M., & Franks, P. (2014). Physical interventions to manipulate texture and tenderness of fresh meat: a review. *International journal of food properties, 17*(2), 433-453.
[5] Benjakul, S., Visessanguan, W., Ishizaki, S., & Tanaka, M. (2001). Differences in gelation characteristics of natural actomyosin from two species of bigeye snapper, Priacanthus tayenus and Priacanthus macracanthus. *Journal of Food Science, 66*(9), 1311-1318.
[6] Chang, H.-s., Feng, Y., & Hultin, H. O. (2001). Role of pH in gel formation of washed chicken muscle at low ionic strength. *Journal of food biochemistry, 25*(5), 439-457.
[7] Connolly, A., Piggott, C. O., & FitzGerald, R. J. (2014). Technofunctional properties of a brewers' spent grain protein-enriched isolate and its associated enzymatic hydrolysates. *LWT-Food Science and Technology, 59*(2), 1061-1067.
[8] Feng, Y., & Hultin, H. O. (2001). Effect of pH on the rheological and structural properties of gels of water-washed chicken-breast muscle at physiological ionic strength. *Journal of agricultural and food chemistry, 49*(8), 3927-3935.
[9] Fu, X., Hayat, K., Li, Z., Lin, Q., Xu, S., & Wang, S. (2012). Effect of microwave heating on the low-salt gel from silver carp (Hypophthalmichthys molitrix) surimi. *Food Hydrocolloids, 27*(2), 301-308.
[10] Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of biological chemistry, 177*(2), 751-766.
[11] Higuera-Barraza, O., Torres-Arreola, W., Ezquerra-Brauer, J., Cinco-Moroyoqui, F., Figueroa, J. R., & Marquez-Ríos, E. (2017). Effect of pulsed ultrasound on the physicochemical characteristics and emulsifying properties of squid (Dosidicus gigas) mantle proteins. *Ultrasonics sonochemistry, 38*, 829-834.
[12] Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. *Journal of Food Engineering, 93*(4), 386-393.
[13] Jemil, I., Jridi, M., Nasri, R., Ktari, N., Salem, R. B. S.-B., Mehiri, M.,… Nasri, M. (2014). Functional, antioxidant and antibacterial

properties of protein hydrolysates prepared from fish meat fermented by Bacillus subtilis A26. *Process Biochemistry, 49*(6), 963-972.
[14] Jiang, S., Ding, J., Andrade, J., Rababah, T. M., Almajwal, A., Abulmeaty, M. M., & Feng, H. (2017). Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics sonochemistry, 38*, 835-842.
[15] Li, G., Sinclair, A. J., & Li, D. (2011). Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. *Journal of agricultural and food chemistry, 59*(5), 1871-1881.
[16] Lin, T. M., & Park, J. W. (1998). Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. *Journal of Food Science, 63*(2), 215-218.
[17] Liu, H., Gao, L., Ren, Y., & Zhao, Q. (2014). Chemical interactions and protein conformation changes during silver carp (hypophthalmichthys molitrix) surimi gel formation. *International journal of food properties, 17*(8), 1702-1713.
[18] Liu, R., Zhao, S.-m., Liu, Y.-m., Yang, H., Xiong, S.-b., Xie, B.-j., & Qin, L.-h. (2010). Effect of pH on the gel properties and secondary structure of fish myosin. *Food chemistry, 121*(1), 196-202.
[19] Najafian, L., Jafarzade, M., Said, M., & Babji, A. S. (2013). Biochemical properties and antioxidant activity of myofibrillar protein hydrolysates obtained from patin (P angasius sutchi). *International journal of food science & technology, 48*(10), 2014-2022.
[20] Noman, A., Qixing, J., Xu, Y., Ali, A. H., Al-Bukhaiti, W. Q., Abed, S. M., & Xia, W. (2019). Influence of Degree of Hydrolysis on Chemical Composition, Functional Properties, and Antioxidant Activities of Chinese Sturgeon (Acipenser sinensis) Hydrolysates Obtained by using Alcalase 2.4 L. *Journal of Aquatic Food Product Technology*, 1-15.
[21] Qiu, C., Xia, W., & Jiang, Q. (2013). Effect of high hydrostatic pressure (HHP) on myofibril-bound serine proteinases and myofibrillar protein in silver carp (Hypophthalmichthys molitrix). *Food Research International, 52*(1), 199-205.
[22] Ru, L., Liu, Q., Xiong, S., Fu, Y., & Chen, L. (2017). Effects of high intensity unltrasound on structural and physicochemical properties of myosin from silver carp. *Ultrasonics sonochemistry, 37*, 150-157.
[23] Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. *Food chemistry, 205*, 43-51.
[24] Sun, Lin, Y.-C., Liu, W.-F., Qiu, X.-J., Cao, K.-Y., Liu, G.-M., & Cao, M.-J. (2019). Effect of pH shifting on conformation and gelation properties of myosin from skeletal muscle of blue round scads (Decapterus maruadsi). *Food Hydrocolloids, 93*, 137-145.
[25] Sun, X. D., & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. *Comprehensive reviews in food science and food safety, 10*(1), 33-51.
[26] Totosaus, A., Montejano, J. G., Salazar, J. A., & Guerrero, I. (2002). A review of physical and chemical protein-gel induction. *International journal of food science & technology, 37*(6), 589-601.
[27] Wang, Y., Zhang, M., Mujumdar, A. S., & Mothibe, K. J. (2013). Quality changes of dehydrated restructured fish product from silver carp (Hypophthalmichthys molitrix) as affected by drying methods. *Food and Bioprocess Technology, 6*(7), 1664-1680.
[28] Wen, Q. H., Tu, Z. C., Zhang, L., Wang, H., & Chang, H. X. (2017). Effect of high intensity ultrasound on the gel and structural properties of Ctenopharyngodon idellus myofibrillar protein. *Journal of food biochemistry, 41*(1), e12288.
[29] Xiong, W., Wang, Y., Zhang, C., Wan, J., Shah, B. R., Pei, Y.,... Li, B. (2016). High intensity ultrasound modified ovalbumin: structure, interface and gelation properties. *Ultrasonics sonochemistry, 31*, 302-309.
[30] Xu, Y., Xia, W., Yang, F., & Nie, X. (2010). Physical and chemical changes of silver carp sausages during fermentation with Pediococcus pentosaceus. *Food chemistry, 122*(3), 633-637.
[31] Yamamoto, K., Samejima, K., & Yasui, T. (1987). The structure of myosin filaments and the properties of heat-induced gel in the presence and absence of C-protein. *Agricultural and biological chemistry, 51*(1), 197-203.
[32] Yu, H. C., & Tan, F. J. (2017). Effect of ultrasonic pretreatment on the antioxidant properties of porcine liver protein hydrolysates. *International journal of food science & technology, 52*(6), 1392-1399.
[33] Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. *Ultrasonics sonochemistry, 34*, 960-967.
[34] Zou, Y., Yang, H., Li, P., Zhang, M., Zhang, X., Xu, W., & Wang, D. (2018). Effect of different time of ultrasound treatment on physicochemical, thermal, and antioxidant properties of chicken plasma protein. *Poultry science, 98*(4), 1925-1933.

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