Effects of Gallic Acid by Ultrasound on Physicochemical Properties of Lateolabrax Japonicas Myofibrillar Protein

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Abstract. The effects of different concentrations of gallic acid (0 mg/g, 1 mg/g, 2 mg/g, 4 mg/g, and 6 mg/g) on the physicochemical properties of Lateolabrax japonicas myofibrillar protein were studied with 400 W ultrasound. The results showed that gallic acid decreased the particle size, total sulfhydryl group, carbonyl and dimer tyrosine content and Ca^{2+}-ATPase activity ($P<0.05$) of myofibrillar protein, however, increased the zeta potential. Ultrasonic wave could cooperate with gallic acid to slow down protein oxidation and make the protein solution system more stable. When the concentration of gallic acid was 2 mg/g, the indicators of protein solution were most favorable, which improved the properties of protein.

1 Introduction

Lateolabrax japonicas (LJ) tastes delicious, fresh and tender. In addition, there are rich amino acids, vitamins and trace elements in LJ [1]. LJ can be eaten fresh, marinated, smoked, or processed into surimi products. However, the myofibrillar protein (MP) directly affects the surimi products quality and flavor [2]. The different processing methods and exogenous additives effect on the physicochemical properties of meat products have studied. Huang et al. [3] showed that the solubility, emulsifying activity, foaming stability of MP decreased under different freezing temperatures and thawing methods. The protein is easily denatured and oxidized during processing, but the single or compound exogenous additives can control the protein oxidation. When sodium alginate is added to MP, the structure of MP is changed, and the viscosity and stability are enhanced [4].

Gallic acid (GA) is a natural phenolic compound containing three phenolic hydroxyl groups. It has good antioxidant properties and can delay the oxidation of proteins [5]. Ultrasound is often used to change the physicochemical properties of food, such as hardness and maturity. There are few reports on the effects of the combination of ultrasonic technology and GA on the MP in aquatic products. The objective of the current study is to determine the effects of ultrasound with GA and improve the stability of protein, which provide the theoretical support for the deep processing of LJ.

2 Materials and methods

2.1 Materials

Lateolabrax japonicas: average body length 30 ± 1 cm, weight 1000 ± 50 g, purchased from aquatic products wholesale market in Keji Road, Jinzhou City, Liaoning Province.

2.2 MP extraction

Referring to the method of Li et al. [6].

2.3 Preparation and ultrasonic treatment of GA MP mixed system

The preparation method of GA MP mixed system refers to the method of Zhang et al.[7]. The content of GA in MP was 0, 1, 2, 4, 6 mg/g, respectively. 400 W ultrasound treated sample for 20 min (water temperature was 4 ºC). After treatment, the samples were stored in a refrigerator at 4 ºC for later use.

2.4 Changes of physiochemical properties

2.4.1 Particle size

According to the method of Shimada et al. [8].

2.4.2 Zeta potential

The Zeta potential of the sample was measured with a particle size analyzer. The measuring temperature was 25 ºC, and the equilibration time was 2 min.

2.4.3 Total sulfhydryl and active sulfhydryl content

On the basis of Youngsawatdigul and Park [9] method, using DTNB method for determination.

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2.4.4 Ca^{2+}-ATPase activity determination

In line with the method of Thanonkaew et al. [10].

2.4.5 Dimer Tyrosine

Referring to Davies [11] method.

2.5 Statistical analysis

Use SPSS19.0 software to analyze and process the experimental data for significance, use Origin8.5 for graphing, and repeat each experiment at least 3 times.

3 Results and analysis

3.1 Particle size

As shown in Fig1, with the GA added increased, particle size trend first decreased, and then increased. When the addition of GA was 2 mg/g, particle size was the lowest. The average particle size of MP was significantly decreased ($P<0.05$) after GA treatment. It indicated that GA could effectively reduce the particle size of protein molecules in the solution and inhibit molecular aggregation. When the ultrasound was 400 W, the average particle size value was lower than that of the non-ultrasonic treatment. This was because the ultrasonic cavitations could cause the force between the particles to break and make the particle size smaller. Zhao et al. [12] proved that ultrasonic pretreatment of milk samples can get the particle size smaller and more uniform, which was consistent with the experimental results of ultrasonic treatment.

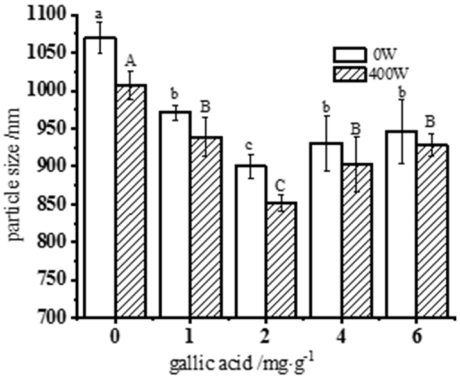

Fig1. Effect of gallic acid on the particle size of MP under ultrasonic treatment

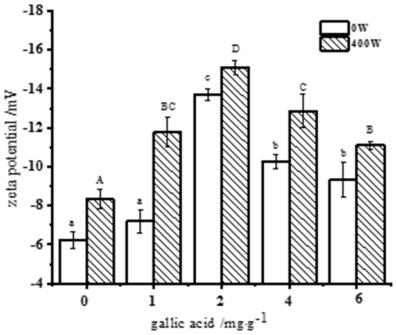

Fig2. Effect of gallic acid on Zeta potential of MP under ultrasonic treatment

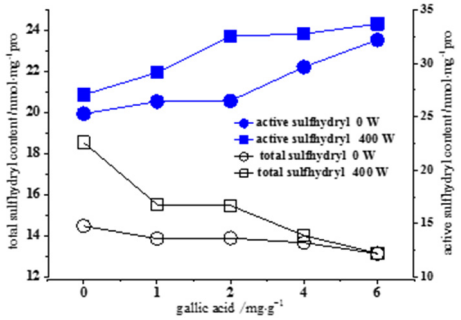

Fig3. Effect of gallic acid on SH content of MP under ultrasonic treatment

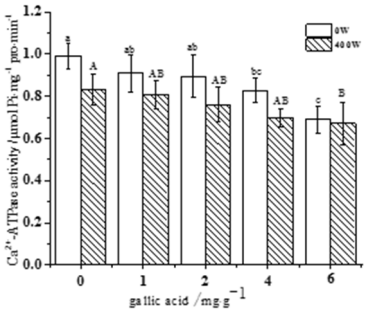

Fig4. Ca^{2+}-ATPase activities of MP under ultrasonic treatment with GA

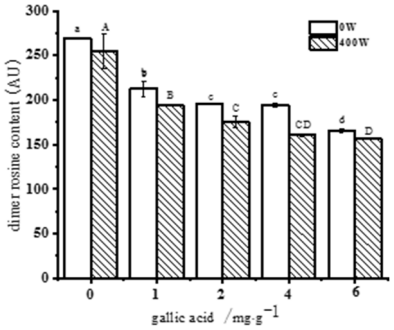

Fig5. Dimer tyrosine content of MP under ultrasonic treatment with GA

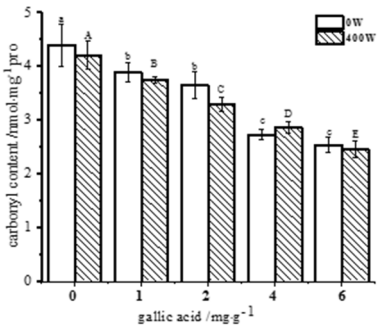

Fig6. Carbonyl content of MP under ultrasonic treatment with GA

3.2 Zeta potential

The zeta potentials of the samples were all negative, which indicated that there were more negatively charged amino acids on the protein surface than positively charged amino acids (Fig2). With the increase of the GA, the potential decreased, and then increased. When GA was 2 mg/g, the potential value of sample was the lowest. The potential value of MP using the 400 W ultrasonic

treatment was lower than that of the non-ultrasound treatment. Zhang et al. [13] indicated that high-intensity ultrasonic waves could destroy protein molecules and expose more surfaces of the protein molecules, increasing the negative charge and strengthening the electrostatic repulsion between particles. Therefore, the ultrasonic synergistic treatment has obvious effect on the stability of the protein solution.

3.3 Total sulfhydryl content and active sulfhydryl content

Compared with MP without GA, GA significantly decreased the total sulfhydryl content of MP ($P<0.05$). But, there was no significantly difference in the total sulfhydryl content of MP between the different concentration treatment groups ($P>0.05$) (Fig3). This was because phenolic substances were prone to oxidate and form quinones, GA was covalently cross-linked with the sulfhydryl groups to generate sulfhydryl-quinone products [14]. As the concentration of GA increased, the content of protein active sulfhydryl groups increased, indicating that polyphenols effectively could slow down the oxidation of protein side chains. GA could protect MP side chain groups. Ultrasound could significantly reduce the total sulfhydryl content, increase the content of active sulfhydryl groups. After ultrasonic treatment, the sulfhydryl groups were exposed from the protein surface. As Amiri [15] showed that 300 W ultrasound performed the beef MP for 30 minutes and obtained the highest active sulfhydryl content.

3.4 Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity can reflect the degree of myosin degeneration. After different concentrations of GA treated with MP, the activity of Ca^{2+}-ATPase decreased (Fig4), because the sulfhydryl groups were oxidized to form disulfide bonds. Among them, the Ca^{2+}-ATPase activity values of the treatment groups with GA of 1 mg/g and 2 mg/g were not significantly different from that without GA ($P>0.05$). When the GA were 4 mg/g and 6 mg/g, Ca^{2+}-ATPase activity was significantly reduced ($P<0.05$). The enzyme activity by ultrasonic treatment was lower than that of without ultrasonic treatment. Because ultrasonic could reduce the content of total sulfhydryl groups, which indirectly led to the decrease of Ca^{2+}-ATPase activity [16]. It demonstrated that the change trend of Ca^{2+}-ATPase activity and total sulfhydryl content was consistent, so the decrease of Ca^{2+}-ATPase activity was related to total sulfhydryl content.

3.5 Dimer Tyrosine content

Tyrosine is an amino acid that is easily oxidized after being attacked by free radicals, then covalently cross-links with other amino acid residues to form dimer tyrosine. With the GA increased, the content of dimer tyrosine decreased significantly ($P<0.05$) (Fig5), showing that GA could reduce the attack by free radicals and make casein the binding between acid molecules blocked. The content of dimer tyrosine with 400 W treatment was lower than that of MP without ultrasonic treatment. The reason was that the ultrasonic waves could disperse the protein molecules, the molecules could not aggregate each other, and the formation of dimer tyrosine was blocked.

3.6 Carbonyl content

In Fig6, after adding GA, the carbonyl content of the treatment group was significantly reduced ($P<0.05$). It suggested that GA could inhibit protein oxidation, scavenge hydroxyl free radicals, prevent the chain reaction of protein carbonyl groups, and ultimately reduce the generation of carbonyl groups. With the increasing of GA, the number of hydroxyl groups also increased, which could increase the cross-linking of GA and protein, so the carbonyl content continued to decrease. The carbonyl content at 400 W was slightly lower than that of the non-ultrasound group. Estévez [17] showed low concentrations of GA and Quercetin can inhibit the formation of carbonyl groups in pork chymotrypsin, which was consistent with the results of the current study.

4 Conclusion

GA decreased the particle size and the activity of Ca^{2+}-ATPase of myofibril protein, but the absolute value of potential increased. In addition, the content of total sulfhydryl, carbonyl and dimer tyrosine was significantly decreased ($P<0.05$). As a natural antioxidant, GA could effectively inhibit the oxidation of aquatic protein. When the concentration of GA was 2 mg/g, the various indicators of the protein solution were most favorable, giving the protein good quality. To a certain extent, ultrasound could cooperate with GA to slow down protein oxidation, improve the parts physiochemical of protein.

Acknowledgements

This work was supported by basic research project of the Educational Department of Liaoning Province, China(LJ2019009).

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