ENHANCED WATER SOLUBILITY OF MYOFIBRILLAR PROTEIN THROUGH DISRUPTION AND INHIBITION OF MYOSIN ASSEMBLY BY ULTRASOUND COMBINED WITH POLYPHENOL MODIFICATION

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I. INTRODUCTION

Because of the rich variety of essential amino acids and the high biological value of muscle-derived proteins, the development of muscle protein beverages as nutritional supplements not only meets the requirements of certain specific groups (e.g., infants, the elderly, etc.) but also promotes the further development of meat. However, a major challenge preventing the further development of the beverages derived from muscle proteins is that myofibrillar protein (MP, about 55%-60% of meat protein), particularly myosin, becomes insoluble in water or low-salt/ionic media [1]. Thus, the aim of this study was to explore the effects of high-intensity ultrasound combined with polyphenol modification on myosin filamentous polymers and the solubility and stability of MP.

II. MATERIALS AND METHODS

Synthesis of MP-EGCG complexes were carried out with different method. For without ultrasound samples, MT: physical mixing treatment (MP+MT). FT: free radical-mediated adduction treatment (MP+FT). AT: alkaline reaction treatment (MP+AT). For ultrasound samples, native MP was first subjected to ultrasound treatment, and followed by grafting of EGCG using different methods following the above procedure (UMP+MT, UMP+AT, and UMT+FT). The native MP without or with ultrasound treatment (MP or UMP) and pH shifting treatment for MP or UMP (MP+pH_{shift} or UMP+pH_{shift}) were used as the control. The conjugation efficiency, total sulfhydryl and free amino content, solubility, zeta potential, particle size, and confocal laser scanning microscopy were measured according to Liu *et al.* [2] and Zhang *et al.* [3]. The data were analysed by Statistix 8.1 software package and reported as the mean ± the standard error. One-way analysis of variance (ANOVA) and Tukey's multiple comparison were used to detect the significant differences ($P<0.05$) among the sample means.

III. RESULTS AND DISCUSSION

As shown in Fig. 1A, ultrasound treatment increased the degree of EGCG substitution, suggesting that ultrasound pre-treatment contributed to more EGCG grafting on the protein molecule. As shown in Fig. 1B-C, a large number of sulfhydryl and free amino were consumed ($P<0.05$), indicating that EGCG was mainly bound to cysteine and lysine residues on the MP. The UMP+AT samples showed the lowest content of sulfhydryl and free amino among all samples ($P<0.05$), demonstrating that the great number of EGCG was bound to the UMP. The native MP had low solubility (2.7%) in water (Fig. 1D). For UMP, the solubility was further increased compared with native MP, and the highest solubility was showed in the UMP+AT sample ($P<0.05$). This may be related to the depolymerization of filamentous myosin and the promotion of more binding sites for capture by EGCG. In this case, electrostatic repulsion and steric hindrance between myosin molecules caused by grafted EGCG would effectively weaken the myosin filament-forming ability, which ultimately increased the solubility of MP in water. As shown in Fig. 1E, the UMP+AT gave higher absolute zeta potential than native MP ($P<0.05$). This suggest that the strong electrostatic repulsion among myosin particles ensured a sufficient intermolecular separation distance and thus greatly increased the dispersion and stability of the UMP+AT samples in water. Native MP exhibited an intact fibrous structure and relatively large

particle size (Fig. 1F and G). Compared to the native MP samples, the UMP-EGCG complexes obtained by AT displayed a considerable reduction in $D_{4,3}$ ($P<0.05$). The decreased particle size favoured water and protein particle interactions and thereby enhancing the solubility of protein in water (Fig.1 E). More so, aqueous solutions containing UMP-EGCG complexes retained a small number of larger particulate species (filamentous fragments) and most of small particle size species (myosin monomers and their dimers and oligomers or small-sized myosin filaments) (Fig. 1G).

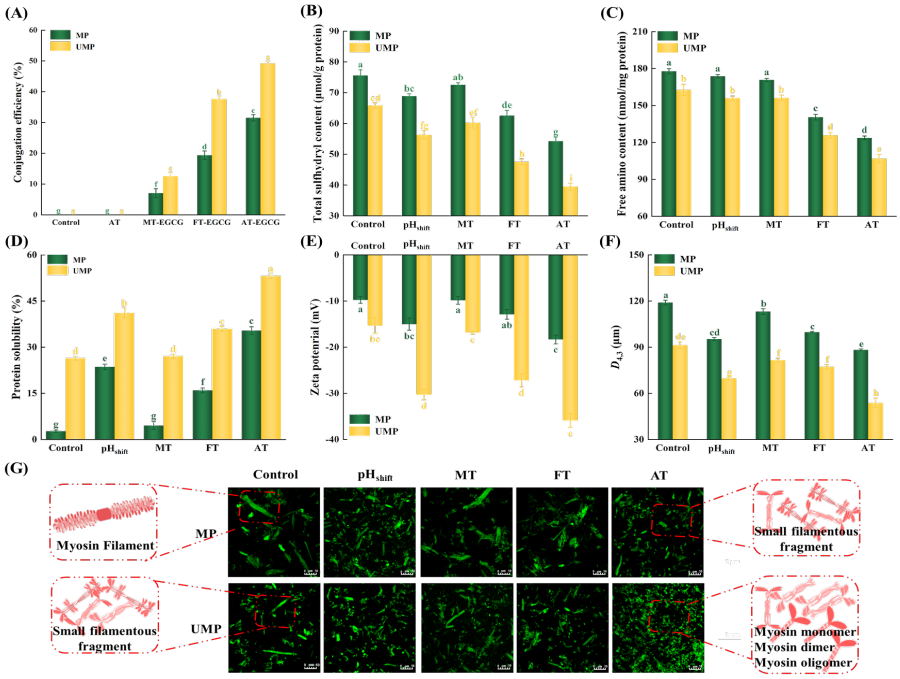

Figure 1. Changes in the conjugation efficiency (A), total sulfhydryl content (B), free amino content (C), solubility (D), zeta potential (E), particle size distribution (F), and confocal laser scanning microscopy micrographs (G) of aqueous suspensions of myofibrillar protein subjected to different treatments. [a-i] Refers to the significant differences among the different groups ($P < 0.05$).

IV. CONCLUSION

The grafted EGCG could improve the solubility of MP in aqueous solution, impair the filament-forming ability to some extent and prevent the formation of filamentous myosin polymers. The best modified conjugates were obtained by combination of ultrasound pre-treatment and alkaline treatment.

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