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# Study of the combined effects of a gelatin-derived cryoprotective peptide and a non-peptide antioxidant in a fish mince model system

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ABSTRACT

The combined effect of a gelatin-derived cryoprotective peptide (Pro–Ala–Gly–Tyr) and a non-peptide antioxidant (caffeic acid) in a fish mince model system was investigated. The physicochemical properties (peroxide value, thiobarbituric acid-reactive substances, total sulfhydryl group content and protein carbonyl content), water distribution and thermal properties of Japanese sea bass mince were determined before and after 6 freeze-thaw cycles. Mince supplemented with a combination of a cryoprotective tetrapeptide (12.5 ppm) and 50 ppm caffeic acid had the lowest peroxide value and comparable thiobarbituric acid-reactive substances to 100 ppm caffeic acid. The cryoprotective tetrapeptide at 12.5 ppm also showed the greatest effect when combined with caffeic acid in lowering protein oxidation, in protecting the total sulfhydryl group content and in having the lowest protein carbonylation, which led to least myosin heavy chain denaturation. The state of water was studied using low-field nuclear magnetic resonance. The tetrapeptide reduced the formation of free water in the mince, especially in conjunction with caffeic acid. Thus, the cryoprotective tetrapeptide worked with caffeic acid in retarding the quality losses in the mince induced by freezing and thawing.



## 1. Introduction

Freezing is the most widely used preservation method to maintain fish quality, accounting for a 50% share of the total processed fish for human consumption and 21% of total fish production (Gonçalves, Nielsen, & Jessen, 2012). The state and mobility of the water in the muscle tissue affects the structure and texture of muscle (Andersen & Rinnan, 2002). To ensure optimal quality, it is necessary that water be frozen quickly to assure small ice crystals and that subsequent storage minimize fluctuations so as to not provide opportunities for water molecules to aggregate which causes the destruction of muscle proteins and loss of functional properties such as the ability to retain water (Andersen & Jørgensen, 2004). Therefore, during storage cryoprotectants have sometimes been added to reduce the denaturation of muscle proteins from ice crystal formation and the increased salt concentration in the unfrozen phase (Benjakul & Visessanguan, 2011).

Beside commercial cryoprotectants (such as a mixture of sucrose and sorbitol) (Liu, Kong, Han, Chen, & He, 2014), some protein hydrolysates have been shown to exhibit a cryoprotective effect without increasing the sweetness of seafood (Cheung, Liceaga, & Li-Chan, 2009; Hossain et al., 2004; Kittipatthanabawon, Benjakul, Visessanguan, & Shahidi, 2012). However, the study of the cryoprotective effect of a pure peptide with a known amino acid sequence, especially in seafood, is very limited. A recent study from this laboratory indicated that the tetrapeptide Pro–Ala–Gly–Tyr isolated from an Amur sturgeon skin gelatin hydrolysate showed a cryoprotective effect in fish mince subjected to different freeze-thaw cycles using low- eld nuclear magnetic resonance (LF $^{1}$H NMR) (Nikoo et al., 2014). Damodaran (2007) suggested that the unique tripeptide repeating structure of –Gly–Pro–X– or –Gly–Z–Hyp– in gelatin peptides might play a role in their ice crystal growth inhibitory characteristic. The tetrapeptide also reduced the formation of thiobarbituric acid-reactive substances (TBARS) in the mince. However, caffeic acid showed a greater effect in preventing lipid oxidation (Nikoo et al., 2014).

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