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# 学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）

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## 学位論文題目

Study on the functional enhancement of fish collagen by glycation using the Maillard reaction

（メイラード反応を用いた糖修飾による魚類コラーゲンの機能性増強に関する研究）

The objectives of this study are to investigate the improving effect of the Maillard reaction on the antioxidant activity and anti-inflammation activity of collagen, to understand the relationship between the functional improving effect and progress of the Maillard reaction. In order to expand the application and proceeding valorization of fish collagen, the author focused on the relation between the enhanced antioxidant activity of collagen with the Maillard reaction proceeding as well as the mechanism of the increased anti-inflammatory of collagen undergoing the Maillard reaction.

This study consists of six chapters: **Chapter I** presented the general introduction of this work; **Chapter II** and **Chapter III** described the enhanced antioxidant and anti-inflammatory activities of tilapia scale collagen by glycation via the Maillard reaction; **Chapter IV** investigated the improved antioxidant and anti-inflammatory activities of glycated sturgeon notochord collagen hydrolysates. **Chapter V** and **Chapter VI** included the general discussion and conclusions.

**Chapter I** presented the background of this study. Concretely, collagen as a crucial protein resource has been applied in various areas, like the food industry and biomedical area. The utilization of collagen extracted from fish processing by-products derived increasing attention because it is consistent with humanity's interest. Additionally, tilapia scale and sturgeon notochord, two typical fish processing by-products, are promising materials for type I and II collagen extraction, respectively. On the other hand, developing and enhancing protein functionality is in the spotlight of the food science area as it fits consumers' demands. Moreover, the Maillard reaction is a brilliant protein modification method to enhance its health-beneficial functions, such as antioxidant and anti-inflammatory activities.

**Chapter II** characterized the glycated tilapia scale collagen and investigated the antioxidant activity of the glycated tilapia scale collagen. Specifically, lyophilized tilapia scale collagen was mixed with a half weight of alginate oligosaccharide (AO) or glucose (Glc) and incubated at 60 °C and 35% relative humidity for up to 18 h to produce the Maillard-type glycated collagen (C-AO and C-Glc, respectively). As glycation progressed, the amount of attached sugar coupled with UV-vis absorbance at 294 nm and 420 nm increased more rapidly in C-Glc than in C-AO, and the available lysine decreased rapidly in C-Glc compared with C-AO. The early-to-middle- and late-stage products of the Maillard reaction were involved in enhanced antioxidant activity of C-AO and C-Glc, respectively. Additionally, C-AO acquired antioxidant activity without marked available lysine loss. Moreover, the cytoprotective effect of collagen in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced cell oxidative

damage was enhanced by glycation, achieved by reducing malondialdehyde (MDA) content and increasing superoxide dismutase (SOD) and catalase activities (CAT).

**Chapter III** investigated the anti-inflammatory activity of glycated tilapia scale collagen. The C-AO and C-Glc were prepared as the previous description, and were employed in the lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophage model to examine the anti-inflammatory activity. The results presented the anti-inflammatory activity of collagen in the reduction of inflammation mediators (TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and NO). Additionally, the anti-inflammatory activity of collagen was enhanced with AO glycation for 18 h (C-AO18h), which was maintained until 24-h glycation. Contrarily, C-Glc shows no enhanced anti-inflammatory activity along all the glycation periods. The enhanced anti-inflammatory activity of C-AO18h was induced by depressed the LPS receptor (*Tlr4* and *Cd14*) and *Myd88* mRNA expression. Moreover, compared with row collagen, C-AO18h treatment significantly up-regulated the activity of endogenous antioxidant enzymes (SOD and CAT) and reduced the MDA level in the LPS-stimulated macrophages. The enhanced antioxidant activity of C-AO18h in the inflammation stage could contribute to the anti-inflammatory activity.

**Chapter IV** explored the extensiveness of applying the Maillard reaction to enhance the health-beneficial functions (antioxidant and anti-inflammatory functions) of collagen in varied reaction conditions. In this chapter, the protein resource was changed from tilapia scale collagen (**Chapter II** and **Chapter III**) to sturgeon notochord collagen-papain-hydrolysates (CP). The glycation condition was changed from 60 °C with 35% relative humidity (**Chapter II** and **Chapter III**) to 80 °C with 75% relative humidity (CP-sugars). In addition, glucuronic acid (GlcUA, uronic acid), a derivative of Glc, was employed as a modified sugar. The reason for the employment of GlcUA is that the carboxyl groups contained in GlcUA and AO were found to contribute to enhancing the anti-inflammatory action. The results of amino acid analysis illustrated that GlcUA had a higher reactivity to the reactive amino acids than Glc and AO. The health-beneficial functions of CP-sugars were evaluated by the ABTS radical scavenging assay and by quantification of inflammatory compound (TNF- $\alpha$  and NO) production in LPS-stimulated macrophages, respectively. Results indicate that the antioxidant and anti-inflammatory functions of CP-sugars were increased with the glycation processes, and AO-glycation enhanced the health-beneficial functions of CP without a marked loss of reactive amino acids compared with the Glc- and GlcUA-glycations. Moreover, contenting of carboxyl groups in reducing sugar is not a determining factor but could contribute to health-beneficial function enhancement during the Maillard reaction.

In conclusion, as described in **Chapter V** (General discussion) and **Chapter VI** (Conclusions and prospective), this study successfully created novel multifunctional fish collagens by glycation with reducing sugars using the Maillard reaction. Additionally, the health-beneficial functions of fish collagen were significantly enhanced by AO-glycation. The AO-glycation displayed function improvements without both excessive losses of lysine and remarkable glycation processing, which reduced the risk of the Maillard reaction application. Moreover, this study proved the extensiveness of applying the Maillard reaction to enhance the health-beneficial functions of collagen-related ingredients in various reaction conditions. The achievements of the present study will contribute to the expand the utilization of collagen-related ingredients as food and biomaterials and to valorize the fish processing by-products.