学位論文内容の要旨

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学 位 論 文 題 目

Study on the enhancement of anti-inflammatory effect
using uronic acid-type glycation in fish protein
(ウロン酸修飾を活用した魚肉タンパク質の抗炎症機能改変に関する研究)

Noncommunicable diseases (NCDs), including obesity, diabetes, cardiovascular disease, and chronic kidney disease, are highly concerned worldwide. The incidence of NCDs is associated with chronic inflammation, and the prevention of chronic inflammation is recognized as one of the major targets for health maintenance. Researchers in the field of health and food sciences recently have promoted the concept of preventing chronic inflammation through diet. Various kinds of components with anti-inflammatory effect have been found in various foods, however, there is little knowledge regarding the anti-inflammatory effect of marine protein. Therefore, development of protein material with anti-inflammatory effect from underutilized marine bioproducts would contribute to increase the added value of marine proteins and to the development of new usage opportunities.

Chum salmon was selected and used as the underutilized marine bioresource in this study. In north Japan, large-scale hatching and stocking projects have ensured a stable supply of chum salmon over the long term. However, the meat quality of overmatured chum salmon deteriorates significantly, and most are discarded because it has low edible value. Even so, the major protein content is almost no different from that during the feeding migration period. Of the meat, myofibrillar protein (Mf) constitutes approximately 55% to 60% of the total meat protein. In addition, as a method, glycation via the Maillard reaction was focused on to impart anti-inflammatory effect to chum salmon Mf in this study.

The Maillard reaction, a complex chain reaction initiated by dehydration condensation between the amino group of protein (mainly the ε-amino groups of lysine residues) and the reducing end of sugar, has a major impact on the quality of food, including changes in physical properties and imparting flavor. Furthermore, it has been reported that the introduction of reducing sugar units to protein using the Maillard reaction is effective in modifying the physiological functions of various food proteins. Therefore, this study focused on applying the Maillard-type glycation to chum salmon Mf to investigate its potential impact on anti-inflammatory effect and to explore its molecular mechanisms for

the functional enhancement.

Chapter 1 investigated the role of carboxyl group in uronic acid in enhancing the anti-inflammatory effect of Mf. Lyophilized Mf was reacted with various reducing sugars at 60 °C and 35% relative humidity through the Maillard reaction. After pepsin and trypsin digestion, the anti-inflammatory effect was evaluated by measuring the secretions of proinflammatory cytokines and nitric oxide in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage. The anti-inflammatory effect of Mf was not affected by glycation with glucose or galactose, whereas it was strongly enhanced by glycation with uronic acid-type reducing sugars: glucuronic acid, galacturonic acid, and alginate oligosaccharide. These results indicate that the presence of carboxyl group in reducing sugar is important for enhancing the anti-inflammatory effect of Mf.

In Chapter 2, the molecular mechanism of the enhanced anti-inflammatory effect of Mf was investigated by comparing the effects of various glycations in Chapter 1 on the secretions of proinflammatory cytokines and LPS-stimulated signaling pathways in RAW 264.7 macrophages. Glycated Mf with uronic acid-type reducing sugars suppressed the expression of *cd14* and further suppressed the expressions of *tlr4* and *myd88* compared with unglycated Mf as well as glycated Mf with glucose and galactose. Furthermore, glycated Mf with uronic acid-type reducing sugars significantly suppressed the expressions and secretions of proinflammatory cytokines. The results indicate that uronic acid-type glycation enhances anti-inflammatory effect of Mf by attenuating the expression of *cd14* and enhancing Mf-induced suppression in the TLR4-MyD88-dependent inflammatory signaling pathway.

In Chapter 3, the efficacy of glycation with edible uronic acid-containing oligosaccharide via the Maillard reaction was investigated for enhancing the anti-inflammatory effect of Mf. Lyophilized Mf was reacted with pectin oligosaccharide (half of the total protein weight) at 60 °C and 35% relative humidity to produce glycated Mf. The available lysine content, the amount of PO bound to Mf, and browning were measured to evaluate the Maillard reaction progress. After pepsin and trypsin digestion, the anti-inflammatory effect was evaluated, and the anti-inflammatory effect of Mf was enhanced with PO-glycation proceeding without marked lysine loss and excess browning. These results indicate that as an edible reducing sugar, pectin oligosaccharide could be an effective bio-industrial material for developing anti-inflammatory Mf.

In conclusion, this study successfully achieved the creation of a fish protein material with an excellent anti-inflammatory effect through uronic acid-type glycation via the Maillard reaction. The findings from this research propose an innovative utilization method that imparts a new function to fish protein. By further advancing the knowledge of this study, it becomes possible to contribute to the enhanced utilization of various underutilized protein resources.