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

Studies on the industrial applications of gelatin and peptide produced from sturgeon by-products

（チョウザメの副産物から得られるゼラチンとペプチドの産業応用に関する研究）

Heads are the primary fish by-products left unutilized in many species, including the commercial fishes in Bangladesh. In this study, I intended to develop the methods to transform fish heads into value-added products using commercial sturgeon heads as a model. I focused on collagen and its derivatives (gelatin and peptides) in this study because fishery by-products are rich in collagen, which is applicable to a wide range of industries.

Firstly, a new method to produce gelatins from the sturgeon head was developed, and the extracted gelatins were characterized. Due to its hardness, the head was heated at 65 °C (3–3.5 h) to separate the tissues. Then, the skin was used to optimize pretreatment and extraction conditions. From the yields and SDS-PAGE analysis of the samples, the optimized pretreatment conditions for type A and type B gelatins were determined as 0.05 M HCl (1 h) and 0.1 M NaOH (1 h), respectively. The best extraction conditions were: (1) for type A gelatin: with distilled water (DW) (w/v 1:5) at 35 °C, pH 7 when stirring at 200 rpm for 3 h, and (2) for type B gelatin: with DW (w/v 1:5) at 50 °C, pH 8 when stirring at 200 rpm for 1 h. After the decalcification of extracted residues with 0.05 M HCl for 3 h, re-extraction of gelatin was possible. The yields (extraction + re-extraction, dry weight gelatin/wet weight sample) of type A and type B gelatins from the mixed tissues (skin, scales, pectoral fins, muscle, bones, gills, and small cartilage pieces) of the head were 5.0% and 7.3%, respectively. The relative gelatin content, estimated from the sample's relative hydroxyproline content to that of the purified collagen, was higher in type A (60.3%) than type B (39.7%). Type A gelatin also showed higher intensities of α- and β-bands of gelatin in SDS-PAGE. Thus, gelatin

purity was higher in Type A. A larger breaking force, lower braking strain, and higher gelling temperature were found in type A. In contrast, type B gelatin had higher emulsion activity and stability and foam expansion and stability. Thus, type A and type B gelatins showed distinct proximate composition and functional properties. The present study suggested that sturgeon head would be a new promising source of gelatin for foods or cosmetics industries.

Secondly, peptides were produced from the sturgeon head and skin through autoclaving, blending, and enzymatic hydrolysis. The yield (dry peptide weight/dry tissue weight) was highest in skin tissue peptides (STP; 77.1%), followed by skin collagen peptides (SCP; 55.4%), and lowest in head tissue peptides (HTP; 41.3%). HTP contained more ROS-scavenging amino acid residues and showed significantly higher $ABTS^{\bullet}$ scavenging activity. HTP also had significant extracellular and intracellular cell-protective activities against oxidative stress. These data strongly suggest that HTP is suitable for cosmetics since the extracellular oxidative stress promotes the aging of our skin. Although the yield is lower in HTP than STP and SCP, the sturgeon head's weight is approximately six times higher than the skin; thus, the head is a novel source of peptides with antioxidant activity. Additionally, the effects of HTP, STP, and SCP on mouse L929 fibroblast activities were examined. All samples significantly increased L929 proliferation and migration, but only SCP and STP activated fibroblasts' collagen production. The potency strongly correlated with collagen content in the samples, suggesting that collagen peptides in the samples have more potent activity than non-collagenous peptides. Thus, the sturgeon skin would be more suitable for producing collagen-rich bioactive peptides. I propose to use SCP and STP for wound healing because fibroblast activation is crucial for wound healing. Since SCP and STP also have antioxidant activity, they are suitable for treating chronic wounds, in which excess oxidative stress inhibits the healing.

Thirdly, a simple and highly efficient method was developed to produce high-quality gelatin useful for tissue engineering. Type A and Type C skin gelatins showed excellent yield (>57%, dry weight gelatin/dry weight tissue) with >97% gelatin content, higher intensity of gelatin bands in SDS-PAGE, and <0.2 EU/mg endotoxin. A simple crosslinking method of type A gelatin gel with 1.0 mM or 5 mM EDC was useful for cell culture. This is the first report for biomedical grade fish gelatin with >97% gelatin content.

Finally, I addressed a general discussion intending to propose an advanced processing model for value-added products from fish fillets and collagen-derived bioactive compounds from

by-products toward zero-discard fish-processing industries in Bangladesh. The market and potentiality of the new products, potential benefits to society, and future perspectives and challenges to realize the proposed model in Bangladesh were discussed. As fish collagen and its derivatives show various health benefits against chronic diseases, and the patients who suffer chronic diseases in Bangladesh are increasing, collagen-rich functional foods would be excellent to overcome these diseases in this country. Some challenges need to be overcome, such as collection, transportation, and storing of the fishes and their by-products throughout this country. Another challenge is the clean and quality by-products with less endotoxin contaminated samples, especially for biomedical-grade products. The Bangladesh government must take necessary actions or help other organizations and companies.

In conclusion, this study will help to develop a new industry of gelatin and peptide from the sturgeon by-products in Japan and create a new research area on the wise-use of fish by-products in Bangladesh. The industrialization of the deep processing of major commercial fishes in Bangladesh would open a novel industrial sector in Bangladesh. Consequently, a new economic sector provides diversified jobs, and the growing national economy will lead to people's better quality of life and livelihoods both in Japan and Bangladesh.