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学位論文内容の要旨

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学 位 論 文 題 目

Studies on the biological activities of mottled skate (*Raja pulchra*) by-products and their possible industrial applications

（カスベ（エイ）加工由来副生物の生物活性およびその産業応用に関する研究）

Cartilage is primarily composed of proteoglycans and collagen. Bioactive compounds derived from animal cartilage have multiple bioactivities and are incorporated in popular health products. The aging population and increases in degenerative and chronic diseases will stimulate the rapid growth of market demand for cartilage products since they show high bioactivities for the treatments of these diseases. Commercial production of the bioactive compounds mainly involves cartilages of mammals and poultry. However, these traditional sources are associated with concerns on zoonoses, such as bovine spongiform encephalopathy and swine flu; and thus, cartilage products from fish processing by-products have gained growing attention because of their high safety and activities. Shark cartilage is the primary source of marine cartilage now. However, shark catches are decreasing worldwide, owing to overfishing. Therefore, this project tried to develop a new cartilage source – skate cartilage.

In northern Hokkaido, skate fishery is one of the essential industries; however, because only the fins of the skate are used for food, a massive volume of waste material is generated by the skate processing industry. The cartilaginous skate head and axial skeleton are rich in chondroitin sulfate (CS) and type II collagen (C-II). The objective of the research in this thesis is to find new bioactivities of compounds contained in the skate cartilage. The skate *Raja pulchra*, which is the dominant species of the skate

fishery in northern Hokkaido, was used as a model species.

The cartilage of *Raja pulchra* contains CS polysaccharides, C-II, non-collagenous proteins, and minerals. First, the anti-obesity functions of CS were investigated *in vitro* and *in vivo*. In this study, CS polysaccharides were obtained after the enzyme hydrolysis of the cartilage and were further hydrolyzed to oligosaccharides. Skate CS has anti-obesity activity. Both CS polysaccharides and oligosaccharides suppressed pancreatic lipase activity as well as proliferation and lipid accumulation in mature adipocytes. Dietary intake of CS oligosaccharides could ameliorate obesity in the high-fat diet mice model. CS oligosaccharides prevented gaining in body weight, liver weight and adipose tissue weight, maintained lower food consumption, inhibited intestinal absorption of triglyceride, and adjusted the serum endotoxin level. The molecular weight (MWt) of the CS affected the CS mode of action: CS polysaccharides had stronger lipase inhibitory activity, whereas CS oligosaccharides suppressed adipocyte lipid accumulation at a lower dose. Thus, a formulation containing both CSs may be more effective as an anti-obesity functional food.

Second, the antioxidant activities of CS and C-II peptides were evaluated. Both C-II peptides and thermally hydrolyzed CS oligosaccharides (CSo) have antioxidant activity. High free radical scavenging activity was observed in the < 3 kDa fraction of the papain-hydrolyzed C-II collagen peptides (Pa-CP) and CSo. The activity of CSo was mainly due to the intermediate and final products of the non-enzymatic browning reaction that occurred during the high-temperature hydrolysis. Furthermore, Pa-CP and CSo did not show cytotoxicity. Taken together, Pa-CP and CSo may act as antioxidants for functional foods owing to their high intestinal absorptivity.

Third, the biological activity in the by-product of skate chondroitin sulfate production (BP-sCS) was found. After isolating CS polysaccharides from the cartilage, the rest of the material is BP-sCS, which contains C-II peptides, non-collagenous peptides, minerals, and a small amount of CS polysaccharides. This study indicated BP-sCS could promote wound healing under oxidative stress. BP-sCS showed free radical

scavenging activity, protected fibroblast from oxidative stress, scavenged intracellular ROS, promoted fibroblast proliferation/metabolism, stimulated collagen synthesis by fibroblasts, and had no adverse effects on fibroblast migration and gel contraction. In addition, BP-sCS counteracted AAPH-induced oxidative stress damage that inhibited fibroblast migration. These effects of BP-sCS were due to cooperation among its ingredient biomacromolecules: type II collagen peptides, non-collagenous peptides, and CS polysaccharides. These findings indicate that BP-sCS could be a novel and superior wound-healing promoter for bioactive wound dressings.

Finally, future tasks for the realization of commercial products from fish cartilage and its significances and influences were discussed. In this thesis, three keys, sustainable skate production, the new fish-processing model, and the market demand, were discussed toward the future realization of more efficient usage of skate cartilage. In northern Hokkaido, skate production increased from the year 2016. Since the low fecundity of skate makes low rates of population increase and low capacity of recovering from overfishing, precise resource management of skate species is required. The present studies also suggest that diversification of skate cartilage products having different functionality is possible and makes the high efficiency of the skate-processing industry. The industrialization of fish-derived cartilage products is beneficial for achieving sustainable development of local economies and society.

In conclusion, the present studies clarified new bioactivities of skate cartilage by-products and assessed their possible applications. Skate by-products are low-cost, environmentally friendly, no contamination with zoonosis, and no religious objections. These advantages indicate that skate by-products could be competitive, valuable products. The present thesis will be beneficial to achieve the zero-discard, highly efficient skate-processing industry that will activate the regional economy in the future.