別紙様式８

研　究　主　論　文　抄　録

論文題目　**Biological extraction of chitin from prawn waste and their utilization by chemical modifications to increase physiological activities**
（カニやエビの殻からの生物学的なキチンの抽出と化学修飾を用いた生理活性向上による有効利用）

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主論文要旨

Biopolymers are synthesized by living organisms that use them in structural and/or physiological support to themselves. Structures of biopolymers are fundamentally based on polysaccharide e.g. cellulose, chitin etc. or protein e.g. collagen and keratin. Chitin is the second most abundant biopolymer after cellulose. Chitin and chitosan are composed of glucosamine and *N*-acetyl glucosamine monomers. Chitosan is deacetylated form of chitin. Chitosan has commonly been used in many industrial areas such as biotechnology, food, pharmaceuticals, and wastewater treatment; however, solubility and viscosity of chitosan pose problems in some applications. In this study, biological treatment of prawn waste for chitin production was investigated firstly and regulation of the physicochemical characteristics as well as physiological activities of chitosan was performed by three types of chemical modification.

Chapter 1 describes the background and the purpose of this study.

Chapter 2 describes the summery of previous researches for utilization of chitin and chitosan and about physiological activities of natural compounds to clarify the methods of this study.

Chapter 3 describes the biological treatment of prawn waste for chitin production. Lactic acid and protease produced in fermentations were used to extract chitin from prawn shell waste. Different strategies were applied by using lactic acid producing bacterium, *Lactococcus lactis*, and a protease producer, *Teredinobacter turnirae* in the presence of various glucose concentrations. In the individual cultivation, *L. lactis* removed the inorganic materials efficiently, while *T. turnirae* performed better in deproteinization process. Cofermentation of both bacteria was also conducted using three different protocols. Highest recovery yield of chitin was observed in one of the protocol where *T. turnirae* was first inoculated followed by *L. lactis* inoculation in 5% glucose medium.

Chapter 4 describes the chemical modification of chitosan by quaternization. Before quaternization, depolymerization of chitosan was achieved by using different amounts of sodium nitrite. Low-molecular-weight (47 kDa) and medium-molecular-weight (198 kDa) chitosans were obtained from high-molecular-weight (544 kDa) chitosan. Antioxidant activities of chitosans were strongly depended on its molecular weight. Antioxidant activities were increasing by decreasing of molecular weight of chitosans. To investigate the effect of molecular weight on physiological activity, chitosan oligosaccharides were used for antioxidant and *in vitro* assays. As expected, chitosan oligosaccharides had the highest antioxidant activities in native chitosan samples. However, increasing molecular weight caused cell death of U937 cells. Depolymerised chitosans reduced triglyceride accumulation but also caused cell differentiation in 3T3-L1 cell line. The quaternization of chitosan was performed in low- and high-molecular-weight chitosans. The quaternization efficiency was increased by decreasing the molecular weight of chitosan. High-molecular-weight chitosan had strong H bonds between amine and hydroxyl groups. Therefore, at the same conditions, modification degree was lower in high-molecular-weight chitosan than low-molecular-weight chitosan.

Chapter 5 describes the modification of chitosan by thiolation. Thiolated chitosan derivatives were prepared by using EDAC covalent connector. The highest grafting ratio of thioglycolic acid was observed at 1 % in low-molecular-weight chitosan, The high molecular weight and high thiol content derivative caused the highest cell death in U937 cells in thiolated chitosans. Increasing concentrations of thiolated chitosans showed increasing cell death.

Chapter 6 describes the modification by grafting chitosan-caffeic acid derivatives. They were also prepared by using EDAC covalent connector in different conditions such as molecular weight of chitosan, molar ratio of chitosan and caffeic acid, reaction temperature, pH, and reaction time. The EDAC showed maximum activity at 3-h, pH 5.0 and room temperature conditions. The products were water-soluble in all pH and showed lower viscosity than native chitosan. The highest grafting ratio of caffeic acid was observed at 15 % in low-molecular-weight chitosan. After 5 % grafting of caffeic acid into chitosan, the grafting efficiency was increased by decreasing molecular weight of chitosan at the same conditions. Caffeic acid modifications showed higher radical-scavenging activity than thiolated chitosans at the same concentrations. Low concentrations of chitosan-caffeic acid derivatives supported cell growth U937. However, increasing concentrations of derivatives showed cell death activity. Generally, high-molecular-weight chitosan-caffeic acid derivatives had higher cell death activity than medium-molecular-weight chitosans.

Finally, Chapter 7 summarizes this study.