博 士 論 文 の 内 容 の 要 旨

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学位授与年月日	2022 年 3 月 20 日
論文題目	Study of molecular size influences on processability and gelation of regenerated silk fibroin aqueous solution （再生シルクフィブロイン水溶液の加工性とゲル化における分子サイズの影響に関する研究）

（博士論文の内容の要旨）

The decrease of molecular weight (MW) of regenerated silk fibroin (RSF) was occurred during degumming and dissolving process. Although influence of MW and the MW distribution on general polymer material processability and mechanical, thermal, and rheological properties have been studied, few reports have described studies investigating the influences of MW and the distribution on silk fibroin material. In those studies, in order to prepare the RSFs with different MW and MW distributions, the decrease MW was induced by changing the degumming or dissolving conditions. However, these SFs are not strictly of the same origin, because the preparation conditions are mutually different. Furthermore, it has been known that the RSF aqueous solution form to gel with long term during storage. For industrial application to produce a silk new material, ensuring of the stability of RSF aqueous solution and elucidation of the gelation mechanism of RSF aqueous solution during storage are important. Gelation mechanisms of RSF has been reported in several reports with focus on changing of secondary structures, but these studies did not investigate gelation mechanisms of RSF aqueous solution on standing without any stimuli during storage, especially with focus on the molecular size.

To prepare SF fractions with different MW, the appropriate conditions for fractionation of RSF by ammonium sulfate precipitation process known as a rapid, mild, inexpensive, and high-yield method were investigated. The MW and the distribution of each fraction were determined by gel permeation chromatography (GPC) and SDS-polyacrylamide electrophoresis (SDS-PAGE). After the formation of films from the fractionated RSF, the secondary structure, surface properties, and cell proliferation on the films were evaluated. Nanofiber nonwoven mats and 3D porous sponges were fabricated from the fractionated RSF aqueous solution by electrospinning and freeze-thaw processing, respectively, and then their structures and mechanical properties were analyzed.

To investigate the influence of MW on the gelation, RSF aqueous solution hydrolyzed by alkali at 60 °C was used. RSF molecular size changes and formation of particles in the aqueous solution during storage were found by GPC and dynamic light scattering (DLS) measurement, respectively. The formation particles of RSF during storage of aqueous solution was confirmed by measurement of viscosity and secondary structure of RSF aqueous solution during storage was determined by Fourier Transform Infrared Spectroscopy (FTIR) measurement.

The results showed that ammonium sulfate precipitation using a dialysis membrane at low temperature is an appropriate fractionation method for RSF. MW of each fractionated RSF was different. Amino acid analysis showed a different composition in RSF of lowest MW fraction comparing with other fractions which have almost same amino acid composition. The secondary structure of cast films formed from the fractionated RSFs were not significantly different. The coating films from the fractionated RSFs showed same zeta potential and cell proliferation, but the coating film of lowest MW fractionation showed slightly greater hydrophobicity than the others by water contact angle measurement. Furthermore, the difference of MW affects the morphology and mechanical properties of the nanofibers and sponges fabricated from fractions, whereas no influence of MW was observed on the secondary structure or crystallinity of the fabricated materials.

GPC analysis of RSF solution at various storage time revealed decreasing molecular size due to cleavage of molecules according to storage time, and rapid decreasing in molecular size was observed at near gelation. On the other hand, no gelation was observed in the hydrolyzed RSF aqueous solution, which contained only lower MW molecules. Particle analysis of the RSF aqueous solution during storage showed the formation of particles with diameter of around 100-400 nm by incubation at 60 °C. Viscosity of RSF aqueous solution decreased with increasing storage time. The secondary structure of RSF freeze-dried the aqueous solution at various storage time was measured by FTIR analysis, and it was found that the ratio of β-sheet structure increased, and the ratio of random and α-helix structure decreased with increasing storage time.

In conclusion, the MW difference of RSF within the range of this study affects to several properties of the fabricated materials from the aqueous solution, but is not a crucial factor to fabricate RSF materials. Gelation mechanism of RSF aqueous solution during storage was thought that RSF molecules are cleaved by hydrolysis in the aqueous solution during storage and the cleaved small molecules are interacted each other resulting in formation particles, and then the particles were aggregated by interaction with large molecular size RSF remained in the aqueous solution and finally gelation was completed.