Studies on the micro-phase separation in mixed carrageenan gels using particle tracking

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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Chapter 1 of this thesis is briefly aimed to introduce the systems and technique used in this study. Biopolymers as food hydrocolloids are generally utilized in food industries to control the texture of food products to meet the sensory preference of targeted costumers. Food hydrocolloids such as carrageenans are commonly used in food industry as rheology modifier i.e. thickener, stabilizer and gelling agent. In addition to pure carrageenans, carrageenan mixtures are also utilized which provides greater controllability of the physical properties and texture. Due to its importance in industries, several studies ranging from macroscopic to molecular level has been performed to understand the gelation mechanism of these carrageenan gels as well as its mixtures. However, the gelation mechanism and network structure at microscopic point of view is still not well understood. In the present study, the gelation mechanism and network structure of carrageenan gels (κ-, ι- and λ-) as well as its mixtures were studied at the microscopic observation using particle tracking. In addition, the phase separated structure of mixture solutions of κ- and ι- and κ- and λ- were investigated.

Chapter 2 describes our developed algorithm to improve the accuracy of particle identification and centroid coordinates for each particle image in particle tracking technique. The algorithm introduced two techniques; 1) cutting off by each threshold at the peak in the pixel intensity distribution for each image of local area around the particle, and 2) calculation of the centroid based on pixel intensities in the original image of the particle instead of binarized data. The former properly cuts the noise in the background for each particle which has large variety in level particle by particle due to fluctuating illuminations and out-of-focus particles in the image, and the latter avoids the loss of accuracy by the commonly used binarization. We have demonstrated that the algorithm significantly improves the accuracy in determination of centroid coordinates and the correctness in particle identification. We have also validated the advantage of the algorithm in accuracy by applying the algorithm to a sequence of confocal microscopy images of diffusing particles in a polysaccharide solution. This algorithm will be significantly useful in particle tracking technique for biological systems, especially for fluorescence microscopy observations with considerable obstructive stray fluorescent signals.

Chapter 3 presents the network structure and gelation mechanism of kappa carrageenan (KC) and iota carrageenan (IC) investigated using multiple particle tracking. Based on bulk rheology measurements, KC gels exhibit characteristics of a hard and brittle gel, whereas IC gels form a soft and so-called “weak-gel”. Particle tracking revealed the differences in the local physical properties of the two gel systems, which are not accessible with the macroscopic rheological measurements. The mean square displacement (MSD) of the probe particles were investigated to characterize changes in the gel network structure on cooling and storage. On cooling, the MSD of the probe particles in the KC solution decreased drastically at around the gelling temperature, demonstrating the trapping of particles within the network structures of KC gels. The MSD of the probe particles in the IC solution exhibited diffusive behavior even far below the gelling temperature on cooling, although the MSD

decreased on storage. These results suggest that KC solutions formed a permanent gel network structure of KC chain aggregates that restricted the motion of particles on cooling. For IC at low temperatures, in contrast, the results suggested two possible structures: 1. clusters of IC chain aggregates, or, 2. a loose network with large pores, which allow the diffusion of particles and lead to weak-gel behavior. The aggregates further aggregate to form a more permanent gel network structure during storage.

Chapter 4 describes the gelation mechanism and phase-separated network structure of mixed κ-carrageenan (KC) and ι-carrageenan (IC) investigated using particle tracking. The thermally driven random motion of fluorescent particles (diameter = 100 nm) was utilized to probe the local circumstances of the gelling carrageenan at the sub-micron scale. The mean square displacement (MSD) was used to characterize the changes in the network structure on cooling and storage for 1 day. The temperature dependence of ensemble-averaged MSD for the particles in the mixture of KC and IC gels showed a two-step decrease on cooling, consistent with the two-step increase in viscoelasticity observed in macroscopic rheological measurements. This result revealed the independent formation of KC and IC chain aggregates in the mixture. The individual MSD of particles in the mixture showed a large distribution after 1-day storage, indicating the emergence of microstructural heterogeneity in the gel. We consider that this heterogeneity came from the frozen structure on the way to phase-separated network structure. The van Hove correlation plots suggested the presence of two groups of particles with fast and slow mobilities in the mixture. Plotting the MSD of individual particles vs α with the MSD scaled as t^{α} and t the lag time suggested a bimodal distribution made up of fast and slow particles. The presence of two groups of particles with different mobilities suggested that mixtures of KC and IC was frozen on the way to a phase-separated network structures made of KC-rich and IC-rich domains with a size of >100 nm due to the network formation of KC and IC chains.

Chapter 5 presents the dynamic rheological measurements and particle tracking's carried out to investigate the gelation mechanism and network structure of mixture solutions of κ- (KC) and λ-carrageenan (LC) gels in macro- and microscopic level using rheometer and particle tracking. Mixtures of KC and LC with a total carrageenan concentration of 1.5% and 15mM of K^{+} were prepared with various mixing ratio. The storage modulus, G' , of KC alone showed the largest value and the G' of the mixtures decreased as the proportion of KC decreases. The mean square displacement (MSD) of particles in the mixtures were used to characterize the changes in the network on cooling and following 1 day storage. On cooling, a single-step decrease in ensemble averaged MSD was observed consistent with the rheological measurement. The individual MSD of particles following 1 day storage showed a large distribution especially for gels with low proportion of KC suggesting a microstructural heterogeneity. This heterogeneity was correlated with the phase-separated network structure made of KC-rich and LC-rich domains. The distribution of individual MSD at 10s and exponent α in the relationship MSD~τ^{α} where τ the lag time revealed a bimodal distribution suggesting two group of particles with inhibit and diffusive mobilities in KC-rich and LC-rich domains. Based on theoretical modelling on the rheological measurements using the Takayanagi blending laws and particle tracking measurements of sub-micron particles, we propose that mixtures of KC and LC demonstrated a phase transition from sea-island to bi-continuous to island-sea network structure.

Finally, we conclude based on the experimental results that carrageenan mixtures formed a phase separate structure. In addition, it was found that the particle tracking is a useful method to elucidate the underlying structure of the gels especially for micro-phase separation.