Water activity dependent glass transition of microorganisms for elucidation of the desiccation tolerance mechanism

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The growth of bacteria becomes negligible under a low water activity (a_w) condition, and thus microbiological hygiene control has been treated as inconsiderable factor for dry foods ($a_w < 0.85$). However, cases of foodborne illness originated from dry foods have been frequently occurred. In our previous studies (in progress), it was suggested that the glass-rubber transition (briefly, glass transition) of bacteria determined by thermomechanical approach is a trigger of bacterial inactive-active transition. Since the mechanical glass transition detected by the approach was a macroscopic phenomenon, more direct information due to molecular dynamics was required to valid the suggestion.

At a constant a_w condition, glass transition temperature (T_g) of food can be controlled by the addition of hydrophilic materials such as sugar. If the bacterial transition can be controlled based on T_g, the efficiency for sterilization will be improved. The purpose of this study was to understand the effect of a_w and additives on the molecular dynamics of bacteria.

Cronobacter Sakazakii was employed as the bacteria sample. Glycerol was used as a typical additive. A large amount of bacteria sample was cultured in laboratory and freeze-dried. The freeze-dried bacteria were mixed with D-glycerol/D_2O, H-glycerol/D_2O, and D-glycerol/H_2O solution to obtain bacteria with glycerol sample (0.2 g-glycerol/g-anhydrous bacteria). The formulations were homogenized by the repetition of dehydration (vacuum-drying at 298 K) and rehydration. The a_w of the bacteria with and without glycerol was adjusted under saturated salts in H_2O and D_2O. Incoherent elastic neutron scan was carried out using AGNES (C3-1-1). The energy resolution was 120 μeV, and covered Q range was 0.20–2.7 Å^{-1} ($\lambda = 4.22$ Å, standard resolution mode). The sample was cooled to 100 K and heated up to 360 K in a step manner. The data were collected every 5 K. From Q-dependence of the elastic intensity, mean squire displacement (MSD) was evaluated by the Gaussian fitting.

The MSD increased gradually with the increase in temperature (Fig. 1). The slope of the linear becomes greater at a certain temperature (dynamical transition temperature, T_d). This means that the MSD of atoms changes in nature from harmonic (solid-like) motion to anharmonic (liquid-like) motion. Comparing to hydrated protein samples, the transition behavior was unclear because various motions activate continuously with the increase in temperature.

At the same a_w condition, the MSD at 298 K for sample with glycerol was higher than that for sample without glycerol. These are opposite results from that of hydrated protein samples. As a possible interpretation, bacteria are covered by cell-membrane, and thus molecular dynamics are different between inter-cellular region and intra-cellular region. Glycerol is non-cell membrane permeable, and thus will have existed at the surface of bacteria; there is no plasticization to intra-cellular materials. From the results, it was suggested that the microbiological inactive-reactive transition may be changed by additives because some types of solute will affect the intra-cellular molecular mobility of bacteria.
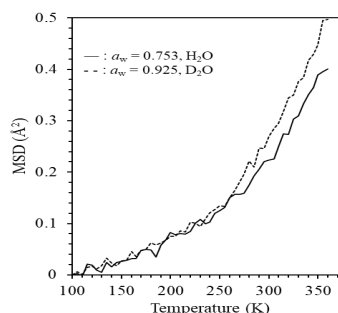

Fig. 1. MSD for *C. sakazakii* samples equilibrated with D_2O.