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Title	Survival strategy of foodborne pathogenic bacteria under low water activity environment: Contribution of glass transition phenomenon of bacterial cells [an abstract of dissertation and a summary of dissertation review]
Author(s)	李, 京珉
Citation	北海道大学. 博士(農学) 甲第14371号
Issue Date	2021-03-25
Doc URL	http://hdl.handle.net/2115/81124
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Туре	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Lee_Kyeongmin_abstract.pdf (論文内容の要旨)



### 学位論文内容の要旨

博士の専攻分野名称:博士(農学)

氏 名: Kyeongmin Lee

#### 学位論文題名

Survival strategy of foodborne pathogenic bacteria under low water activity environment: Contribution of glass transition phenomenon of bacterial cells (低水分活性環境における食品媒介食中毒細菌の生存戦略: 細菌細胞のガラス転移現象の寄与)

Drying is one of the effective techniques of food preservation and it is widely used in the world for long period of time. Dry stress suppresses microbial metabolic activity by reducing free water in foods that is available for microorganisms. In particular, bacteria are known that it is difficult to keep activity in low water activity ( $a_w$ ) foods, although foodborne illness caused by dried foods have been occurring worldwide. The reason why bacterial cells survive under low  $a_w$  environment has not been unclear. To clarify the cause of resistance of pathogenic bacterial cells to low  $a_w$  stress, this study focused on glass transition phenomenon, which is changes in physical properties of bacterial cells, instead of conventional biochemical approaches.

# 1. Relationship between glass transition temperature and heat tolerance in *Salmonella enterica*

Salmonella enterica is a foodborne pathogenic bacterium that has been reported in various dried foods in the world. To clarify the cause of the survival of *S. enterica* in dried foods under low  $a_w$  environment, glass transition phenomenon that is a state change in substance was focused in this study. Five kinds of *S. enterica* serotype under different water activity conditions were examined for the glass transition temperature  $(T_g)$  by using newly developed thermal rheological analysis method. Under low  $a_w$  conditions ( $a_w < 0.75$ ), every *S. enterica* serotypes showed a relatively high  $T_g$  such as 45 - 62°C. These results suggested that *S. enterica* can be present in the glassy state under room temperature and/or below in dried foods. Furthermore, thermal tolerance of dried *S. enterica* in different  $a_w$  levels was examined at 60°C for 10 min. The results demonstrated that the glassy state and the thermal resistance are related to each other, as

S. enterica under low  $a_w$  conditions showed higher thermal resistance. These results would be the basis of understanding for survival of S. enterica in dried foods

#### 2. Strain variability in glass transition temperature of Cronobacter sakazakii

Cronobacter sakazakii is a widely known pathogenic bacterium that contaminate powdered infant formula all over the world. However, the reason for the contamination and survival of *C. sakazakii* in powdered infant formula with low  $a_w$  has been unclear for long time. Because the glass transition phenomenon of bacterial cells might be attributed to the survival of *C. sakazakii* under low  $a_w$  condition, six strains of *C.* sakazakii were examined for determination of  $T_g$ . The results illustrated that there are significant differences in  $T_g$  of *C. sakazakii* among the six strains. However, the  $T_g$  of *C.* sakazakii regardless of strains was ranging from 35 - 60 °C under low  $a_w$  conditions ( $a_w$ < 0.57), which suggests that *C. sakazakii* would exist in a glass state at room temperature and/or below. Survival of *C. sakazakii* in infant formula would be attributed to the glass transition of bacterial cells.

## **3.** Effects of drying methods of *Cronobacter sakazakii* on the survival during storage and the thermal tolerance

Although there are some drying methods for producing dried foods, the effect of drying methods on the survival and the thermal tolerance of dried bacterial cells has not been focused. However, it would be critical for food industry to identify the effect of drying method on the bacterial survival during storage under low  $a_w$  conditions. To clarify the survival behavior of *C. sakazakii* under low  $a_w$  conditions, comparison of the  $T_g$  of air-dried and freeze-dried bacterial cells and investigation of the survival kinetics during storage were conducted. The  $T_g$  of *C. sakazakii* depends on drying method and the air-dried *C. sakazakii* exhibited higher  $T_g$  than that of freeze-dried cells. In addition, the air-dried *C. sakazakii* cells showed strong viability for a long period of time during storage than that of freeze-dried ones. Furthermore, the air-dried *C. sakazakii* cells demonstrated higher survival rate during thermal treatment at 40°C than those of freeze-dried ones, because the  $T_g$  of air-dried *C. sakazakii* was higher than 40°C in contrast to the lower  $T_g$  (ca. 33°C) of freeze-dried *C. sakazakii*. These results suggest that the  $T_g$  of bacterial cells would play a key role in ensuring microbial food safety of dry foods.