Studies on the color improvement technique & mechanism with high ZnPP-forming bacteria in dry-cured meat products

（乾塩漬食肉製品における高ZnPP形成細菌による色調改善技術とメカニズムに関する研究）

Abstract

Nitrite/nitrate has been added to improve the color of meat products, but it is pointed out that they could cause a carcinogenic effect. On the contrary, ZnPP is a main natural red pigment found in dry-cured meat products without nitrate/nitrite. Since some bacteria can stimulate to form ZnPP, they might be contributed to improve the color of meat products without nitrate/nitrite. It was hypothesized that some edible bacteria might be useful to improve the color of meat products by producing ZnPP. Moreover, it was also hypothesized that high ZnPP-forming bacteria promoted the endogenous ZnPP formation in meat. Thus, this study was undertaken to search for edible bacteria that can stimulate ZnPP production in meat products and also to clarify the formation mechanism of ZnPP by bacteria in meat products.

Bacterial isolates were searched from various sources and screened with modified standard plate count agar containing 3% salt and at pH 5.5 under anaerobic conditions. Total 126 isolates were assessed in the aseptic meat homogenate model system we established to check their ZnPP-forming ability. Only three edible bacteria *Lactococcus lactis, Leuconostoc mesenteroides*, and *Enterococcus faecium* were identified from the 44 high ZnPP-forming isolates with 16S rRNA partial gene sequencing. Then high ZnPP-forming edible bacteria were inoculated in aseptic salted minced meat, after incubation a brighter red color and a higher amount of ZnPP was observed in the inoculated groups than non-inoculated control. Furthermore, after heating, the color of inoculated minced

meat was persisted to a degree. Finally, dry-cured sausages were prepared by using these bacteria to check their ZnPP-forming ability meat products. After ripening, a higher amount of ZnPP was observed in the inoculated sausages than that of the control group and the color was almost similar to the nitrite group. Since these bacteria were proven to increase the microbiological safety by the suppression of pathogenic bacteria, it could be concluded that the screened bacteria are promising candidates as multi-functional starter cultures to improve the color of dry-cured sausage by forming ZnPP.

Regarding ZnPP formation mechanism by bacteria, initially, the ZnPP-forming ability of the bacteria was observed in commercial media in the presence of myoglobin, and meat components responsible for ZnPP formation. Next, the ZnPP-forming ability of the bacteria was checked in pre-heated meat and two components which deactivate at 50 and 70°C respectively are necessary for ZnPP formation, and the component that deactivates at 50°C was supplemented by ZnPP-forming bacteria. The ferrochelatase (FECH) activity of bacteria was confirmed by the FECH inhibitor in pre-heated meat and the bacterial metabolites also have the ability to form ZnPP. Moreover, ZnPP-forming bacteria did not degrade myoglobin and hemoglobin. When the meat homogenate was fractionated, only the FECH-containing fraction increased the ZnPP formation in a dose-dependent manner, and FECH was localized in the inner and outer membrane of mitochondria. It was clarified that not only FECH from bacteria but also their metabolites assist ZnPP formation in the meat-inherent mechanism.

The present study confirmed that *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *E. faecium* as high ZnPP-forming bacteria capable of forming ZnPP in dry-cured sausages and improves the color of sausage. High ZnPP-forming bacteria can facilitate the ZnPP formation in meat as FECH suppliers but cannot produce ZnPP by themselves.