Development of koji using new raw materials and comprehensive analysis of active ingredients
（新規原料を用いた麹の開発とその有効成分の網羅的解析）

広島大学大学院統合生命科学研究科
生物工学プログラム　中川拓郎

Chapter 1: Preface

"Koji fungi (koji-mold)" is a "national fungi", and koji-mold is a general term for molds that include *Aspergillus oryzae*, *A. sojae*, and *A. luchuensis*. Since ancient times, Japan have used these koji-molds to make koji from grains and use it in various traditional fermented foods. The most important role of koji in the production of these fermented foods is the supply of enzymes for the degradation of raw materials. Furthermore, enzymes of koji-mold are substrate specific and secreted for degrading raw materials. In recent years, the fermented food trend has brought back attention to traditional foods such as amazake and shio-koji, and various foods have been tried to be "koji-made". These are made possible by the characteristics of koji-mold, which secretes enzymes suitable for the degradation of raw materials and cannot be achieved by yeast alone. In this study, I focused on egg as a new koji material. Eggs are a nutrient-rich food, and there are a variety of recipes around the world. On the other hand, the high-quality nutritional components are only taken as they are, and there is still room for improvement, such as the enzymatic conversion of microorganisms. Therefore, growing koji-mold on eggs was expected to give flavors that cannot be achieved by cooking, such as decomposing egg proteins to produce peptides and amino acids to enhance umami or decomposing lipids to enhance aroma components.

Chapter 2: Preparation of egg-koji for developing a novel food

Chapter 2 consists of egg-koji development method that uses only eggs and koji-mold by selecting and combining cooked egg powder (CEP) and *A. oryzae* AO101 as the most suitable combination. To suppress the explosive growth of harmful bacteria, I improved the sterilization method, watering method, and amount of water. In addition, it was found that egg-koji has a characteristic enzyme activity balance, in which amylase activity is extremely low and protease activity at pH 6 was high compared to grain koji, such as rice and barley. Egg-koji might produce enzymes suitable for taking in nutrients when growing into CEP and would be expected to give a flavor that could not be achieved by cooking or additive.

Chapter 3: Effect on egg component profile in koji

In Chapter 3, I investigated the novel functionality of egg-koji by metabolome analysis using GC-MS. Compounds extracted from steamed CEP and egg-koji with methanol aqueous solution were measured by GC-MS, and the measurement data was subjected to principal component analysis, suggesting that the component profiles of steamed CEP and egg-koji were significantly different. Therefore, it was inferred that the egg-derived components were converted into new substances by koji-mold. Principal component analysis between egg koji prepared with different koji species suggested the formation of specific components by koji-mold. In particular, the metabolite profile of *A. sojae* AS309 clearly separated from other species in the first principal component.

Chapter 4: Egg-koji enhances the richness and umami taste of whole egg

In Chapter 4, I focused on the components related to "richness" among the component changes caused by the fermentation of whole eggs with egg-koji, and investigated their effects on sensory characteristics. Using AO101 as a standard strain of egg-koji, the sensory characteristics of whole egg liquid digests (WELDs) prepared with egg-koji and other grain koji were compared. E-101 (WELDs prepared with egg-koji AO101) was the only WELD that enhances the umami and richness without losing its egg flavor. Additives such as monosodium glutamate and nucleic acid are added to foods to enhance the umami taste, but by using WELD such as E-101, these seasonings and additives can be reduced. Using egg-koji prepared by the different *Aspergillus* strains, WELDs with enhanced umami and richness while retaining the egg flavor of whole eggs could be prepared. Different characteristics of each egg-koji were revealed, such as that E-309 (WELDs prepared with egg-koji *A*. *sojae* AS309) produced more free amino acids and aroma components and E-434 (WELDs prepared with egg-koji *A. luchuensis* AL434) had the potential to take the emulsifying power of free fatty acids, suggesting that seasonings and additives can be reduced in food design. These outcomes have opened the possibility of new egg-related foods.

Chapter 5: Conclusion

Compared to grain (plant) raw materials, animal-derived raw materials tend to contain less carbohydrates and more proteins and fats (lipids), so they retain their unique flavor and physical properties, and it was thought that koji making was difficult until now. Abundant nutrient sources are more susceptible to bacterial contamination, so scaling up production is often an issue. In food development, there are not many examples of consistent development from the lab level (research) to actual production, and I was able to obtain important knowledge of foods using koji, a new raw material. I hope that the findings of this study will serve as an important foundation for future research and development of koji and new materials koji.

参考論文

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