主論文の要約

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学位論文題目

Study on Potential Allergenicity of *TERASI*, Indonesian Shrimp Seasoning,
and its Reduction by Processing Technology

(インドネシア産エビ調味料テラシのアレルギー誘発性と
加工技術による低減化に関する研究)

The objective of this study is to investigate the potential allergenicity of *Terasi,* Indonesian fermented shrimp seasoning and discussing the processing technology for the reduction of the IgE-binding ability of *Terasi* final products. The PhD thesis consists of four chapters including one chapter overall explaining this study and three chapters presenting results and discussion of the research.

Chapter 1. (Introduction). *Terasi* is a popular seasoning of Indonesian traditional fermented shrimp paste with unique flavor and excellent nutritional value, which made by mixing dried small shrimp with salt (10–15% w/w). The mixture is grinded and spread out on the ground to dry with sunlight. The salted shrimp meat is solid and subjected to ferment in anaerobic condition at ambient temperature (25–28 ℃) for at least 3 days or longer until the unique aroma has fully established. It is reasonable to regard *Terasi* as a significant component of human diet and nutrition worldwide. However, shrimp, the raw material of *Terasi* contains allergenic protein, tropomyosin (TM), which is a common allergen of invertebrates, such as crab, squid, octopus, and shellfish. Several studies have been reported that proteolysis due to food fermentation reduced food allergenicity, but no investigation about the potential allergenicity of Terasi.

Chapter 2. The study evaluated the safety of *Terasi* (Indonesian fermented shrimp paste), the product characteristics and allergenicity of 20 types of Indonesian commercial Terasi (CT) that meet the Indonesian National Standard were evaluated with a focus on the major shrimp allergen TM. Marked protein hydrolysis of shrimp muscle occurred in all CT samples, and no protein fragments or specific reaction of anti-TM IgG were observed in SDS-PAGE and immunoblot assays. In a competitive enzyme-linked immunosorbent assay using shrimp allergenic patient sera, it was observed a markedly diminished specific IgE reaction of CT compared with that of shrimp muscle, whereas the IgE-binding ability remained in all CT samples. No clear correlation was found between the degree of protein hydrolysis and IgE reactivity. These results indicate that CT could be defined as a low–allergenic processed seafood but has the possibility to be a causative food for shrimp allergy. Direct immunological

evaluation is required establishing the food safety of CT, because assessments of protein profiles and hydrolysis are not useful for determining the safety of *Terasi*. Moreover, among CTs, raw materials and protocol used are less informed; therefore, the following chapters discuss their potential.

Chapter 3. This study examined the effect of the *Terasi* manufacturing process on the loss of the allergen TM and its IgG/IgE-binding ability. *Terasi* was produced from three shrimps, Akiami (*Acetes japonicus*), Okiami (*Euphausia pacifica*), and Isazaami (*Neomysis awatchensis*). Protein degradation and TM IgE-binding activity were examined by immunoblotting using anti-TM rabbit IgG and competitive enzyme-linked immunosorbent assays using shrimp-allergic patients' sera. TM in the materials was degraded during the manufacturing process, and the IgG-specific response in Akiami meat disappeared at the second fermentation step but remained in both Okiami and Isazaami *Terasi*. In contrast, TM IgE-binding ability of TM in all shrimp meats decreased gradually with the progress of the manufacturing process and nearly disappeared in Akiami *Terasi* (AT), indicating Terasi can be recognized as a low allergenic seafood. However, in order to ensure food safety of *Terasi*, an effective mean for decreasing IgE-binding ability of the final product should be introduced to the *Terasi* manufacture, because progress of the IgE-binding loss varied depending on raw materials.

Chapter 4. As countermeasures against the technical issues raised in Chapter 3, the backslopping method was applied to the *Terasi* manufacture, and its contribution to reducing the potential allergenicity of *Terasi* was examined. That is, three kinds of starters, the low allergenic commercial Terasi as CT, AT (produced in Chapter 3) and HAT (heat treated AT) were added to manufacturing process of the Isazaami *Terasi* which highly remained IgE-binding ability. This chapter demonstrated that backslopping method using *Terasi* products is an effective manner to produce low allergenic *Terasi* by inducing reduction of IgE-binding ability of TM. Addition of the starter accelerated the fermentation of the raw material, effectively promoting the degradation of the shrimp protein and the reduction of the IgE-binding ability of TM. However, the backslopping effect was dependent on the type of *Terasi* used as a starter, and the commercial *Terasi* used in this study did not contribute to the allergenicity reduction of the final product. Interestingly, *Terasi* added as a starter would have acted primarily as a nutrient source to promote microbial fermentation rather than as a source of fermenting microorganisms and endogenous proteases.

Conclusively, *Terasi* manufacture is an effective manufacturing process to reduce the IgE-binding ability of TM and to ensure the low allergenicity potential. *Terasi* can be recognized as a low allergenic seafood when produced under an appropriate manufacturing condition. It is probable that the measurement of the whole protein hydrolysis is effective for allergenicity evaluation of *Terasi* under certain conditions, and it could be used as a screening index. However, analysing IgE-binding activity of the final products is the most important manner to estimate *Terasi* as a low allergenic seafood. It should be noted that the backslopping method using *Terasi* final products could contribute to improving food safety of *Terasi* by reducing the IgE-binding ability of the final products. I believe that these research results will definitely help ensuring food safety of *Terasi*.