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アイゴ Siganus fuscescens臭気の生成メカニズムと臭気の低減法に関する研究

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博士学位論文内容要旨
Abstract

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論文題目 Title	Study of the unpleasant smell in rabbit fish *Siganus fuscescens*: the generation mechanism and method for removal (アイゴ *Siganus fuscescens* 臭気の生成メカニズムと臭気の低減法に関する研究)		

Rabbit fish *Siganus fuscescens* is a common herbivorous marine species distributed in Japan and can be caught off shore around the year. The fish is unpopular and has low commercial value due to the unpleasant smell in its tissues. The excessive consumption of macroalgae by its overpopulation has been considered as one of the significant causes involved in the reduction of seaweed beds, which seriously occurred in coastal areas of Japan. Therefore, this research aimed to develop the method for preventing or removing the unfavorable smell in rabbit fish meat for its wider utilization in food manufacturing, which may be a potential method to manage and restore seaweed beds.

In order to develop the method for removing or preventing the unpleasant smell, the information about the volatile compounds associated with smell and their generation mechanism is needed. Therefore, in chapter 3 of this study, the key volatile compounds contributing to the unpleasant smell in rabbit fish tissues were determined using solid-phase microextraction-gas chromatography-mass spectrometry technique (SPME-GC-MS). The lipid profiles in the tissues of rabbit fish were examined in chapter 4. In chapter 5, the enzymatic generation of the volatile compounds via lipid oxidation model of crude enzyme extracted from rabbit fish viscera and PUFA, were investigated. Lastly, the method for removing the off-odor in rabbit fish meat were developed in chapter 6.

The volatile compounds in rabbit fish muscle, viscera, skin and stomach contents were determined using SPME-GC-MS in chapter 3. Quantities of each volatile compound were estimated by internal standardization method using cyclohexanol. Key volatile compounds that contribute to the overall smell of sample were described by odor activity values (OAV). The highest number and concentration of volatile compounds were observed in viscera, followed by stomach contents, skin and muscle. The key volatile compounds contributing to the overall smell of the meats were hexanal and 1-octen-3-ol, which could be classified as volatile lipid oxidation products (VLOP) from PUFA. Various types of carbonyls, alcohols, amines and sulfur-containing compounds were found in another 3 tissues. The highest level of OAV in these 3 tissues were found at 1-octen-3-one and 1-octen-3-ol, suggesting their strong association with the overall smell of whole fish.

Because the key odor compounds in rabbit fish were VLOP, lipid profiles in rabbit fish tissues were examined in chapter 4 with a special focusing on polyunsaturated fatty acid (PUFA). Crude lipids were extracted from rabbit fish tissues, then converted to fatty acid methyl ester (FAME) and 4-dimethyloxazoline (DMOX) derivatives. Fatty acid composition and their quantitation were determined by analysis of FAME and DMOX using GC and GC-MS. The results presented that rabbit fish lipids contained high levels of PUFA, especially arachidonic acid (ARA), which differed from other carnivorous and omnivorous marine species that consist of mainly docosahexaenoic acid (DHA) and icosapentaenoic acid (EPA).

Lipoxygenase (LOX) has been well known as enzyme that plays important role on the smell development in plants and animals. This enzyme is capable to catalyze the oxidation of PUFA to produce conjugated unsaturated fatty acid hydroperoxides. The breakdown of these unstable compounds forms lower-molecular weight secondary products, which are responsible for the smell generation process. The results from previous chapter led to the possibility that rabbit fish tissues may also contain LOX, which involved in the formation of

volatile compounds associate with the unpleasant smell in its tissues. Therefore, lipid oxidation model of crude enzyme extract and PUFA were used to investigate the generation of volatile compounds products in chapter 5. Viscera, which has the strongest smell, and the highest numbers and concentrations of volatile compounds among other tissues, was selected to be used for crude enzyme preparation. Lipid oxidation model mixtures were prepared by mixing crude enzyme extracted with commercial PUFA, including ARA, linoleic acid (LA), DHA and EPA in sodium phosphate buffer (pH 7.4). The generation of volatile compounds in reaction mixtures were determined immediately without incubation and after 24 hours of incubation at 30 °C in the dark using SPME-GC-MS techniques as describe in chapter 3. A total of 36 and 26 compounds were observed in headspace of reaction mixture of crude enzyme containing n-6 PUFA and n-3 PUFA, respectively. Most of these compounds have been already reported as the compound generated by LOX in various fish species. The results indicated that crude enzyme extracted from rabbit fish viscera contained LOX. The activity of LOX in this study was shown as relative activity (%) calculated from the total amount of volatile compounds generated enzymatically from the breakdown of PUFA. Highest level of LOX activity ($p<0.05$) was clearly observed on ARA. The presence of various 5-, 6- and 8-carbons compounds, such as 1-penten-3-ol, hexanal, 1-octen-3-ol and 1-octen-3-one, in reaction mixture containing crude enzyme extract and PUFA, suggested that the type of LOX in rabbit fish viscera were probably mainly 12- and 15-LOX. Volatile compounds enzymatically generated from the breakdown of PUFA in this study were also similar to the compounds that observed in rabbit fish tissues. Therefore, it could be concluded that rabbit fish viscera LOX initiated the oxidation of PUFA, especially ARA, and led to the formation of VLOP that associate with the unpleasant smell of rabbit fish.

Washing, an essential method used for removing the water-soluble protein and other impurities to concentrate the myofibrils in the fish protein gel production, has been reported to affect the overall smell of fish mince by washing away the off-flavor or promoting the release of other volatile compounds. Moreover, introducing antioxidants in washing solution has also became a successful method to prevent the lipid oxidation during surimi processing and storage. Therefore, in the last chapter, the effect of washing on the quality of rabbit fish meat during washing and storage was investigated with special focus on the removal of volatile compounds. Rabbit fish meat was minced and washed 3 times with 3 volumes of cold (4 °C) distilled water or 0.5% sodium ascorbate solution. Washed meats were then stored at 4 °C in polyethylene bag in the dark for 2, 4 and 6 days. The change of VLOP in the meat during washing and storage were determined using SPME-GC-MS. Compared with unwashed meat, hexanal and 1-octen-3-ol levels in both water- and sodium ascorbate-washed meat significantly decreased ($p<0.05$), but no significant difference in the levels of these 2 compounds could be observed between the meats washed with two different solutions. However, during storage, VLOP from both n-3 and n-6 PUFA were generated only in the meat washed by water, indicating that adding sodium ascorbate as an antioxidant in washing solution could prevent the lipid oxidation and minimize the development of VLOP in rabbit fish washed meat during storage. These suggested that washing is an effective method to remove the key odor compounds associated with the unpleasant smell in rabbit fish washed meat and also able to prevent the generation of those compounds during cold storage, which may lead to the increase of their potential use for manufacturing.