

Study of the unpleasant smell in rabbit fish *Siganus fuscescens*: the generation mechanism and method for removal

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Summary

Rabbit fish (*Siganus fuscescens*), a herbivorous marine fish with a low commercial value due to its unpleasant smell, has been considered as one of the significant causes involved in the reduction of seaweed beds, which seriously occurred in coastal areas of Japan as well as in warm temperate waters around the world. Moreover, the limited fishing of this species consequently has the negative impacts on the productivity of the Japanese shoreline because of the imbalance in fish stock. Therefore, this research aims to develop the method for preventing or decreasing the unfavorable smell in rabbit fish meat to its wider utilization for food manufacturing, which may be a potential method for managing and restoring 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. The volatile compounds in rabbit fish muscle, viscera, skin and stomach contents were determined using solid phase micro-extraction (SPME) gas chromatography mass spectrometer (GC-MS). The key volatile compounds contributing to the overall smell of rabbit fish muscle were hexanal and 1-octen-3-ol, which could be classified as volatile lipid oxidation products from polyunsaturated fatty acid (PUFA). Various types of carbonyls, alcohols, amines and sulfur-containing compounds were found in another 3 tissues. The highest

level of odor activity value (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. Furthermore, after storage the fish meat at 4 °C for 6 days, the meat that was stored as whole fish deteriorated more rapidly than stored as fillets, suggesting the importance of early evisceration on the smell quality of rabbit fish.

Because the key odor compounds in rabbit fish were volatile lipid oxidation products from PUFA, lipid profiles in rabbit fish tissues were examined with a special focusing on 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. Rabbit fish lipids contained high levels of both n-3 and n-6 PUFA, especially n-6 PUFA arachidonic acid (ARA), which differed from other carnivorous and omnivorous marine species that consist of mainly n-3 PUFA, docosahexaenoic acid (DHA) and (e)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 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. 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 results indicated that crude enzyme extracted from rabbit fish viscera may contain LOX because various volatile compounds associated with the fresh fish

smell, such as 5-, 6-, 8-carbon compounds were generated from the reaction with PUFA. These compounds were also similar to the key odor compounds in rabbit fish tissues. Highest level of LOX activity ($p < 0.05$) was clearly observed on ARA than EPA and DHA. The result suggested that the presence of the unpleasant smell in rabbit fish was probably due to LOX in its tissues, which was capable to catalyze the oxidation of PUFA, especially ARA, and led to the formation of volatile lipid oxidation products that contribute to 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 become a successful method to prevent the lipid oxidation during surimi processing and storage. Therefore, 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. Washing rabbit fish mince by both water and 0.5% sodium ascorbate solution could remove the concentration of 2 key odor compounds associated with the unpleasant smell in rabbit fish, including hexanal and 1-octen-3-ol. Moreover, the generation of volatile lipid oxidation products during cold storage could be prevented by adding 0.5% sodium ascorbate as an antioxidant in washing solution. These suggested that washing with antioxidant solution is an effective method to remove the key odor compounds associated with the unpleasant smell in rabbit fish meat and also able to retard the generation of those compounds during cold storage.

Washing method developed in this study is expected to be an effective method for removing the unpleasant smell in rabbit fish meat, which may lead to the increase of their potential use for manufacturing, and be a successful method for managing and restoring seaweed beds.