Summary of Doctoral Thesis

Enrollment year: 2023 y 9 m
UGAS Specialty: Bioresources Science
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Title	Effect of shell shape, post-harvest rearing, and super-chilling storage on the changes in biochemical properties of Pacific oysters (Crassostrea gigas)

Introduction and purpose

Pacific oyster (*Crassostrea gigas*) is a highly significant economic species in the seafood industry and ranks among the world's important aquatic products. According to the report of Food and Agriculture Organization (FAO, 2018), the global production of oyster was 643,500 tons. In recent years, the increasing demand for oysters, particularly in the live and half-shell markets, has been driven by the proliferation of oyster bars. However, like other perishable seafood, oysters have a limited shelf life due to the growth of microorganisms and the enzymatic activity of endogenous proteolytic agents. Therefore, ensuring oyster freshness and achieving optimal quality, especially for raw consumption, becomes crucially important. Many studies reported that superchilling is a highly effective technique for preserving the quality of food. However, few studies focused on the impact of supchilling storage on shelled oysters. Moreover, initial vitality and shell shape of oyster will also affect its shelf life and quality. Therefore, viability recovery and shape selection were also crucial problems to be solved. On the other hand, many studies have confirmed that there are two adenosine monophosphate (AMP) decomposition pathways in oysters and the accumulation of inosine monophosphate (IMP) is different from different conditions of oysters (whole body and separates). Hence, one of the purposes of the study was to clarify the effect of shell shape, post-harvest rearing, and super-chilling storage on the changes in biochemical properties of Pacific oysters. Another objective was to examine the cause of IMP accumulation in the adductor muscle of oysters.

Materials and methods

Live cultured triploid Pacific oysters (*Crassostrea gigas*) were collected from a cultured company in Toyama Prefecture. Oysters were artificially purified by placing them in Deep Ocean water for 48h. Deep Ocean water is seawater with extremely high purity below 300 meters. The water temperature is stable at a low temperature of around 1-2℃ all year round. Oysters are purified by ensuring that clean Deep Ocean water is circulated in the oyster's body. After purification, the oysters were transported to the lab within 48h. The content of ATP-related compounds, AEC value, pH, free amino acids, glycogen, microbial analysis, arginine phosphate and sensory evaluation were used in this study.

Results

The quality changes of shelled Pacific oysters were examined in relation to the effects of superchilling storage at -1 °C for 28 days by measuring changes in biochemical properties

(microbial analysis, ATP-related compounds, pH, free amino acids) and sensory evaluations in Chapter 1. The results indicated that microorganism growth was significantly inhibited during superchilling storage. Adenosine diphosphate (ADP) and AMP accumulated while ATP rapidly decreased in the adductor muscle. Adenosine triphosphate (ATP) and ADP were the primary components in the other 3 tissues including mantle, gill, and body trunk of oysters, and they remained relatively stable over time. The pH and adenylate energy charge (AEC) in the adductor muscle could be utilized as freshness indicators for shelled oysters. However, there were no significant differences ($P > 0.05$) among the free amino acids during whole storage. According to the sensory evaluations, oysters could remain alive and maintain their quality up to 21 days when stored at -1 °C. Therefore, superchilling storage at -1°C could be more effective in preserving eating quality of shelled oysters, allowing for an extension of the shelf life up to 21 days.

Because the level of vitality significantly influences the shelf life of shelled oysters stored at super-chilling temperatures, Chapter 2 investigated the impact of short-term rearing on oyster vitality prior to preservation. Some vitality indexes (pH, AEC value, arginine phosphate and glycogen) in the adductor muscle of oysters were studied after short-term rearing and during storage at -1℃. The results indicated that, after short-term rearing the vitality of oysters recovered in a better condition, as the AEC value, pH and arginine phosphate of short-rearing groups were higher than no rearing group at day 0. The 3-day rearing group showed the highest level vitality among the three groups. During the storage, the pH and AEC values of 3-day rearing group were kept in a high level compared to other two groups. However, there were no significant differences observed in glycogen levels among all the groups. Therefore, it can be concluded that short-term rearing effectively enhances vitality, positively impacting the quality and freshness of Pacific oysters.

Based on the above results, the viability of oyster played an important role in shelf life under superchilling storage. The shell shape of oysters was also strongly correlated with vitality. In Chapter 3, we proposed a non-destructive and real-time classification method for assessing the vitality maintenance ability of Pacific oysters solely based on their external shape features using 3D morphometric techniques. Additionally, this chapter explores the differences between flat and round oysters during superchilling storage. The results showed that the classification accuracy of

round and flat oysters was 70.0% by using 3D morphometric techniques. During superhcilling storage, the round type oysters showed higher vitality than flat type. The AEC value of round type oysters was consistently in the range of 50%-60%. In the flat type, AEC values decreased from 59.19% to 39.98% during the superchilling storage. The results of AEC values also correlated with the intervalval water content. The pH of two groups also showed a similar trend. However, no significant difference of major free amino acids between two types was observed in our experiments. The results suggested that 3D morphometric measurement techniques can have a significant impact on branding and enhancing the quality of oysters. It enables the non-destructive and real-time classification of oysters based on their vitality throughout the supply chain.

Furthermore, in Chapter 4, the regulatory mechanism of IMP formation in the oyster adductor muscle was investigated. Through this study, we confirmed that IMP generation in the oyster adductor muscle under different conditions, primarily due to, the extracellular fluid in oysters.

In the extracellular fluid of oysters, AMP is rapidly broken down, resulting in the generation of IMP. Similarly, IMP is quickly decomposed during incubation. However, when EDTA is added, the decomposition of AMP into IMP occurs in the absence of bivalent metal ions in both the adductor muscle group and extracellular fluid group. While the activity of AMP deaminase in the extracellular fluid remains unaffected, the decomposition of IMP slows down. Under natural conditions, the adductor muscle of oysters is influenced by the extracellular fluid, preventing the significant accumulation of IMP. Removing bivalent ions not only inhibits the adenosine (AdR) pathway in the adductor muscle but also retards the breakdown of IMP by the extracellular fluid.

Conclusion and consideration

To conclude, the shell shape, post-harvest rearing, and super-chilling storage on the changes in biochemical characteristics of pacific oysters were elucidated. This research was expected to be applied to high quality shelled oyster preservation and transportation in the future. Meanwhile, through the investigation of formation of IMP in oyster adductor muscle, this study will contribute to the control of flavour quality of oysters in the storage process.

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e.g. chapter-by-chapter summary in short sentences

Summary paragraphs (introduction, main body and conclusion) in short sentences

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