Summary of Doctoral Thesis

Enrollment year: 2017 y 10 m

UGAS Specialty: Bioresources Science

Name Huamao Wei

Title	Changes in Biochemical Properties and Microstructure of Scallop (*Patinopecten yessoensis*) Striated Adductor Muscle during Freeze-thawing, Freeze-drying and Rehydration Process

Scallop (*Patinopecten yessoensis*), which is a very important economic species as seafood, is one of the important aquatic products in Japan. According to the report of Ministry of Agriculture, Forestry and Fisheries (MAFF), the productions and the export of scallop was 388 kilotons (up 38% than last year) and 84 kilotons (up 76.6% than last year) in 2018. One of the challenges in market distribution is to maintain scallop quality while maximizing its shelf life. Freezing and drying are good ways to maintain the quality of scallops. Many studies reported fish which are frozen in pre-rigor have a better quality than that of in rigor or post-rigor stage. On the other hand, freeze-drying as a good method, which does not cause shrinkage or toughening of the material being dried, and flavors and smells generally remain unchanged, was also widely used. However, few basic information regarding the effect of thawing process on the biochemical changes of pre-rigor scallop adductor muscle after freeze-thawing, freeze-drying and rehydration process is available. Moreover, many studies have confirmed that there are two AMP decomposition pathways in scallops and the accumulation of IMP is less due to the low activity of AMP deaminase. But the studies which focus on AMP decomposition pathway and its influencing factors in scallops are scarce. Therefore, one of the purposes of the project was to clarify the changes in biochemical properties of scallop adductor muscle during freeze-thawing, freeze-drying and rehydration process.

Another aim was to investigate the AMP decomposition pathway in scallop adductor muscle and its influencing factors, such as EDTA or EGTA addition, heating, metal ions concentration change, et al.

In Chapter 1, the effects of thawing methods on the biochemical properties and microstructure of pre-rigor frozen scallop striated adductor muscle was examined. Postmortem biochemical properties (pH, salt solubility, Ca^{2+}-ATPase activity, ATP-related compounds) and microstructural changes in the striated adductor muscle of pre-rigor frozen scallop were studied after thawing and during storage at 4°C. Four thawing methods were used: running water (18°C, R); ice-water (0°C, I); air (4°C, A) and ice-saltwater (-2°C, S). The pH values and salt solubility of R group were lower than the other three thawing groups while I group was highest after thawing. However, no significant difference ($p > 0.05$) in Ca^{2+}-ATPase activity were detected among 4 groups. The microstructure results indicated that the structure of I group was close to that of fresh scallop. Moreover, ATP decomposition rate was the slowest. Therefore, ice-water thawing is the best method because it induced the least changes in the biochemical properties and microstructures of scallop adductor muscle.

Because the ATP decomposition rate in scallops after thawing is much faster than in fresh products, in chapter 2, condition-dependent adenosine monophosphate decomposition pathways by endogenous enzymes in striated adductor muscle from scallop was investigated. The purpose of this study was to confirm inosine monophosphate (IMP) generation and to clarify the decomposition pathway of adenosine monophosphate (AMP) by investigating the properties of AMP, IMP and adenosine (AdR) decomposition enzymes in scallop. The results showed that IMP accumulated due to AMP decomposed by endogenous enzymes in scallops when stored at both 4°C and 20°C. The AMP decomposition rate was highest in the supernatant of homogenized scallop adductor muscle,

follows are the suspended solution and precipitate, while IMP could not be decomposed in scallop. The results indicated that the activity of adenosine deaminase was very high, and this enzyme was involved in an intracellular process in scallop. Moreover, one minute of heating exerted little influence on the AMP and AdR decomposition rates, while 5 min of heating induced enzyme denaturation. The IMP generation rate increased dramatically in scallop crude enzyme solution containing 5 mM EDTA. This suggests that the major pathway of AMP decomposition might change with variations in metal ion concentrations in Japanese scallop.

In chapter 3, based on the above results, the effect of EGTA addition and different ions on the pathways of AMP decomposition in scallop was further studied. Scallop adductor muscle tissue solution was divided into control group (without any treatment) and CP group (containing 0.1% chloramphenicol). These two groups were dialyzed at 4℃ and then incubated at 25℃. Changes in ATP-related compounds of scallop adductor muscle tissue solutions with 5 mM EGTA, 10 mM K^+, 1 mM Ca^{2+} and 6 mM Mg^{2+} additions during incubation were detected by HPLC, and changes in protein composition of both groups were examined by SDS-PAGE during dialysis and incubation period. The results indicated that IMP generated rapidly in scallop crude enzyme solution containing 5 mM EGTA within 2 h. For the control group, the protein in the scallop enzyme solution gradually denatured due to microbial activity during incubation, while CP group did not. Adenosine deaminase and adenosine kinase were detected in denatured protein fraction (40 kDa) by LC-MS/MS. The results suggested that K^+ promoted the decomposition of AMP to IMP, Mg^{2+} promoted the decomposition of AMP to AdR, and Ca^{2+} slightly inhibit the decomposition of AMP.

Furthermore, in Chapter 4, Changes in biochemical propertied and microstructure of Japanese scallop adductor muscle during storage, freeze-drying and rehydration process was investigated. The

biochemical properties (ATP-related compounds, salt solubility, Ca^{2+}-ATPase activity) and microstructural changes in freeze-dried scallop adductor muscle of pre-rigor Japanese scallops during room temperature (18±2℃) storage and rehydration process were studied. The results showed that ATP and ADP contents were maintained or kept at 3.63±0.20 μmol/g and 2.27±0.23 μmol/g in freeze-dried scallop which stored at low humidity (less 10%) and room temperature for 30 days and with no significant difference ($p > 0.05$), and AMP and IMP contents were detected less than 0.42 μmol/g and 0.1 μmol/g, respectively. ATP was decomposed rapidly within 1.5 min of rehydration, accompanied by the accumulation of AMP. As the rehydration time increased, AMP was gradually decomposed into HxR and Hx. Salt solubility of freeze-dried scallop was lower than that of freeze-thawed scallop. However, no significant difference ($p > 0.05$) in Ca^{2+}-ATPase activity of freeze-dried scallop were detected during storage and rehydration process. Digestion results suggested that the rod of myosin was unstable after freeze-drying process while S-1 part was stable, compared with fresh scallop and freeze-thawed scallop. Moreover, the microstructure results indicated that the Z-line of scallop was broken during freeze-drying process.

Note 1 The summary will be published online. Please confirm that UGAS has your permission to do so.

Note 2 In the summary, please do not include any content beyond your control (e.g. patents or intellectual assets) which will make online publication impossible.

Note 3 The summary should be four or five pages in total.