学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）　　氏名：Matthura Labaiden

学 位 論 文 題 目

Elimination of foodborne pathogens from oysters using electorolyzed seawater

（食中毒原因微生物を対象とした電解海水によるカキの浄化）

Shellfish farming is a growing industry around the world but “Shellfish-Borne Disease” controls remain a challenge. Raw or lightly cooked consumption of shellfish including the most cultivated species, oysters, may pose a risk to human health. Although a variety of methods such as UV irradiation, ozonation and chlorination have been developed to reduce bacterial and viral load in water for aquaculture, on-site application of such complex methods in Thailand is still difficult. As an alternative, a combination some of these methods with cheaper and less toxic options such as the use of electrolyzed seawater, and high pressure processing should be considered. This study aimed to determine suitable combination of methods for the elimination of *Escherichia coli* and *norovirus* (NoV) from oysters.

Chapter I focused on the determining a suitable process for the elimination of *E. coli* from oysters using electrolyzed seawater. From the results, available chlorine levels of 0.5 mg/L and above were toxic to oysters. However, *E. coli* elimination from oysters was achieved using electrolyzed seawater at a chlorine concentration 0.3 mg/L, for 24h. It has been reported that electrolyzed seawater is not mutagenic if available chlorine levels are below 2.0 mg/L, and it has a sufficient disinfectant effect level of at available chlorine levels of 1.0 mg/L. Eectrolyzed seawater is valuable for elimination of *E. coli* in oysters.

Chapter II focused on the survivability of NoV under inactivating conditions and in marine environments. Due to the fact that NoV has never been cultured *in vitro* yet, detections of virus particle are still difficult to be in practical uses. Therefore, *feline calicivirus* (FCV) was used as a NoV surrogate and the survivability of FCV was studied under various inactivating conditions. These conditions are UV irradiation, electrolyzation and high pressure processing (HPP). Subsequently, the survivability of NoV as a FCV in seawater and oyster homogenate were estimated. The results showed a 99.9% reduction in infectious titers of FCV by a UV irradiation dose of 25 mJ/cm^2. When electrolyzation of seawater (ESW) was used to disinfect seawater, concentrations of 0.23 and 0.41 mg/L of chlorine, produced by electrolysis of 3% NaCl solution and seawater could reduce infectious titer of FCV more than 99% in 1 min. Though electrolyzed seawater may contain organic halogen compounds like bromo-form, amounts are less than the limit for drinking water standards in Japan and the U.S. if electrolyzation is performed within the range of normal use. Viral infectivity of FCV treated with HPP in this study showed that FCV was inactivated by HPP at 200 MPa after 5 min. Moreover, HPP treatment at 200 MPa does not change the quality of oysters both texturally and visually. Oysters can be separated from their shells under a pressure of 80 MPa for 5 min at 40°C. One-$\log_{10}$ reduction was observed under a pressure of 80 MPa. Furthermore, 1.8-$\log_{10}$ reductions were observed at 40°C for 5 min. Combined heating to 40°C and a pressure of 80 MPa for 5 min, led to reductions of 2.7-$\log_{10}$ without affecting the texture of the oysters. These results

on survivability in marine environments showed that viral titer of FCV was decreased rapidly above 20°C treatment and it is postulated that when water temperature is above 20ºC the infectivity of NoV is likely to be below the detectable limit within 2 weeks. These results indicates that eliminate of NoVs could be achieved after 2 weeks depuration under virus-free environment. Disinfected seawater can be obtained by UV disinfection or ESW even though coastal area are contaminated with NoVs. These results also indicated that NoV does not survive for a long period of time unless temperatures are low. This observation is similar to results from observations of other enteric viruses. This may explain why outbreaks caused by NoV are much more prevalent in the winter than in the summer. Results also showed that, FCV was more stable in sterilized seawater or PBS than untreated seawater. This might be due to the presence of antiviral-substance-producing microorganisms in seawater.

In conclusion, this study demonstrates that ESW at a chlorine concentration 0.3 mg/L, for 24 h is an effective method in *E. coli* elimination in oysters. Furthermore, elimination of NoVs from oysters should be done by using UV irradiation at a dose of 25 mJ/cm^2 or electrolyzed seawater at a chlorine concentration 0.4 mg/L combined with shucking oysters at a pressure of 80 MPa, 40°C for 5 min. From all of the results indicated that ESW is the most suitable and effective method for Thai farmer to eliminate *E. coli* and NoVs from oyster because ESW provides high cost performance and low maintenance.