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Effects of electron beam irradiation on murine norovirus-1 in abalone (*Haliotis discus hannai*) meat and viscera

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ABSTRACT

Many shellfish-borne viral outbreaks have been reported in East Asia, where abalone is predominantly eaten raw. This study investigated the effects of electron beam (e-beam) irradiation (1–10 kGy) on the inactivation of murine norovirus-1 (MNV-1), as a surrogate of human norovirus (NoV). The cell culture lysate of MNV-1 and abalone meat and viscera were irradiated with e-beam (1, 3, 5, 7, and 10 kGy). The titer of MNV-1 significantly ($p < 0.05$) decreased by 0.38–2.18, 0.31–1.45 and 0.41–1.56 $\log_{10}$ PFU/mL in the suspension, abalone meat, and abalone viscera, respectively, as the dose of e-beam irradiation increased. However, all Hunter colors, five sensory attributes and four textures parameter not significantly ($p > 0.05$) different in abalone after e-beam irradiation. D-values were calculated using first-order model and corresponded to 3.92, 6.26, and 5.23 kGy in the suspension, abalone meat, and abalone viscera, respectively. There were no significant changes in food qualities of pH, moisture contents and TBARS levels. Thus, e-beam can be used as an effective and useful non-thermal treatment to reduce MNV-1 in abalone. We suggest using a dose of e-beam irradiation >6.26 kGy to achieve 90% $\log_{10}$ reduction of MNV-1 in abalone without deleterious changes of food qualities.

1. Introduction

Human norovirus (NoV), a small structured RNA virus within the genus *Norovirus* of the family *Caliciviridae* (Noda, Fukuda, & Nishio, 2008), is currently considered the most important human viral pathogen causing acute gastroenteritis. NoV transmission primarily occurs through the fecal-oral-route either by intakes of contaminated water and food, especially bivalve shellfish, or through person-to-person contact directly from food handler during food processing and/or food serving (Rodriguez-Lazaro et al., 2012). However, there is no cell-line for NoV, therefore, murine norovirus-1 (MNV-1) is considered a suitable surrogate model for studies of NoV because of the similarities in the genetic organization that MNV-1 genome identifies the three open reading frames (ORF) characteristic of NoV and a common fecal-oral transmission route (Karst, Wobus, Lay, Davidson, & Virgin, 2003; Wobus, Thackray, & Virgin, 2006).

Consumption of shellfish, especially bivalves such as oysters and clams, has increased considerably during the past 3 decades as well as infectious shellfish-borne outbreaks. In the 1990s, NoVs were the primary viral pathogens associated with shellfish-borne gastroenteritis in the United States. Since then, many studies have confirmed the association between shellfish (especially bivalve molluskan shellfish such as oysters, cockles, and mussels) and NoV infection (Potasman, Paz, & Odeh, 2002; Prato et al., 2004; Shieh et al., 2000).

Abalone is a highly prized marine gastropod mollusk belonging to the genus *Haliotis* of the family *Haliotidae* considered a palatable seafood widely cultured in Australia, China, Japan, Korea, Mexico, South Africa, and the United States (Gordon & Cook, 2001). It has been used as a valuable food source because of its high content of vitamin B_1, vitamin B_2, calcium, phosphorus, and taurine (Kim et al., 2006). In Korea, not only the protein-rich body part of abalone but also the viscera are eaten as sashimi (raw) or marinated with soy sauce, and used as stamina food (Koh, Kim, Cho, Kang, & Kim, 2009; Li, Kim, & Kang, 2014). Traditionally, it has been consumed for postnatal care, skin care, and overall improvement of health condition (Lee et al., 2010).

Recently, Park, Bae, and Ha (2015) suggested a Weibull model of heat inactivation of MNV-1 in abalone meat and viscera where it was simulated when MNV-1-inoculated abalone porridge was

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cooked as thermal food processing. Nevertheless, it is necessary to perform further studies to find means to inactivate NoV. In case of improperly cooked seafood, especially shellfish, or after exposure to contaminated water, the risk of infection is considered high (Lees, 2000). Thus, further processing may be required to improve the safety of raw seafood for human consumption (Jung et al., 2009).

Grove et al. (2015) reported that MNV-1 readily transferred from the knife blade or cutting board to lettuce during the chopping of Romaine lettuce. There is a high probability of contamination during the cooking or preparing process of abalone contaminated by seawater or from the hand of an infected person. Nevertheless, efficient procedures for eradicating viruses from shellfish are not commonly used. Some factors can cause unsanitary conditions such as open or no packaging, and cross-contamination from hand to abalone during cooking and serving. Potasman et al. (2002) highlighted that only two of the diseases transmitted by shellfish are currently preventable by vaccination: hepatitis A virus and poliovirus infection. For this reason, only precautions are adopted to control NoV infection. Abalone meat and viscera are predominantly eaten raw in Asian countries, especially in Korea and Japan (Gao, Tashiro, & Ogawa, 2002; Porturas, Ushio, Watabe, Takada, & Hatae, 1993). Hence, non-thermal process to control NoV while maintaining the freshness of abalone without thermal process is urgently and highly required.

As raw fishery food is eaten after only the washing step in the restaurant or at home, if chemical washing agents are not removed completely or are remained some part of them, it can cause deterioration of flavor and odor of the product can occur. Thus, non-thermal physical sanitization such as high-pressure processing (HPP), pulsed electric field (PEF), ionizing radiation, and ozone are preferred than chemical sanitation for raw seafood processing against NoV, minimizing quality changes in food (Arvanitoyannis, Stratakos, & Mente, 2008; He, Adams, Farkas, & Morrissey, 2002; Khadre & Yousef, 2002).

Electron beam (e-beam) is an ionizing radiation, characterized by a stream of high-energy electrons (Miller, 2006). The United States Food and Drug Administration (US FDA) approved the maximum radiation absorption dosage of 4.5 kGy for uncooked meat, meat byproducts, and certain meat food products, and of 1.5–3.0 kGy for raw packaged poultry. In addition, the absorption dosage for control of Vibrio bacteria and other foodborne microorganisms in or on fresh or frozen molluskan shellfish was established not to exceed 5.5 kGy (21 CFR Part 179.26, 2005). However, the efficacy of e-beam irradiation against foodborne NoV in a diverse range of raw abalone products has not been reported yet. We hypothesized that e-beam irradiation (1–10 kGy) can be used on the inactivation of NoV in abalone *(Haliotis discus hannai)* meat and viscera as an effective non-thermal processing. The objective of the present study was to control NoV contaminated in raw abalone by e-beam. We used MNV-1 as a NoV surrogate to experimentally contaminate abalone *(Haliotis discus hannai)* meat and viscera.

2. Materials & methods

2.1. Cell culture

MNV-1 was maintained in the RAW264.7 cell line (murine macrophage) purchased from the American Type Culture Collection (Rockville, MD, USA). RAW264.7 cells were grown in Dulbecco's minimum essential medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 44 mM sodium bicarbonate (Sigma), and seeded into 75-cm^2 culture flasks for incubation at 37 °C in a humidified incubator (Vision Scientific Co., Seoul, Republic of Korea) containing 5% CO_2. The cells were subcultured every 2 or 3 days. The cell line was cultured by scraping to allow the detaching. MNV-1 was provided by Dr. Skip Virgin, Washington University.

2.2. Virus preparation

When the RAW264.7 cell monolayers achieved 90% confluence in a 175-cm^2 tissue culture flask, the growth medium was aspirated completely, and the monolayers were washed with Dulbecco's phosphate-buffered saline (DPBS; Sigma) pH 7.3. A 3-mL aliquot of the MNV-1 suspension was inoculated in the flasks and incubated at 37 °C with 5% CO_2 atmosphere for 1 h to allow virus adsorption. Then, 35-mL of the maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate) were added to the flasks and incubated at 37 °C in 5% CO_2 atmosphere for 3 days. If more than 90% cytopathic effects were observed, the virus-infected flasks were frozen and thawed 3 times. The viruses were released by cell lysis. The contents were centrifuged at 2500 × *g* for 10 min to remove cell debris, and the supernatant was subsequently harvested. The viruses were stored at −80 °C until further use.

2.3. Sample preparation and inoculation

Abalone was purchased from the local market in Anseong and freshly shucked with a sterilized spoon. The meat (mussel) and viscera from the whole abalone were aseptically removed using a sterile knife and forcep. Abalone meat was cut vertically (2 mm in thickness), and abalone viscera was minced. A 5 g of both samples were placed in 50-ml tube, separately. A 200 μL aliquot of MNV-1 (7–8 $\log_{10}$ PFU/ml) was inoculated in 5.0 g of abalone meat and viscera. The inoculated samples were placed at 3 °C for 24 h. A 1 mL of MNV-1 suspension which was prepared as described in Section 2.2 (6–7 $\log_{10}$ PFU/mL) was put into sterile 1.5 mL tube.

2.4. E-beam irradiation

Samples were irradiated with 1, 3, 5, 7, or 10 kGy of e-beam using a high-energy 10-MeV linear accelerator (MV10-8/635; Mevex Corporation, Ottawa, Canada) at the Seoul Radiology Services, Co., Ltd. (Eumseong-gun, Chungbuk, Republic of Korea). Defined dosages were delivered by conveying the samples across the incident e-beam using a commercial-scale computer processor-controlled conveyor system. Speeds of the conveyor belt used to achieve target radiation dose were 1.52 m/min with 1.1 kW power for 1 kGy and 1.10–3.69 m/min with 8 kW power for 3–10 kGy.

2.5. Virus recovery

After irradiation, MNV-1-contaminated abalone meat and viscera were soaked in 5 mL of DPBS in a 50-mL conical tube. Samples were vortexed for 1 min and shaken in a shaking incubator (Vision Scientific Co.) at 300 rpm for 1 h to elute the virus. After centrifugation at 10,000 × *g* for 30 min at 4 °C, the supernatants were serially filtered using 5.0, 1.2, 0.8, and 0.45 μm filters (Sartorius, Goettingen, Germany). Each eluted solution (viral suspension) was serially diluted 10-fold in DMEM. The virus infection titers were analyzed with a plaque assay. MNV-1 inactivation was calculated as a D-value (e-beam radiation dose needed to achieve a 1 $\log_{10}$ reduction of infectious virus titer for each abalone sample). For the MNV-1 activity, three samples (n = 3) of the abalone meat or viscera for each dose of e-beam irradiation were assayed in triplicate. Non-irradiated samples inoculated with MNV-1 were designated as controls. Virus was also recovered from these control abalone samples.