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Application of gamma irradiation for the reduction of norovirus in traditional Korean half-dried seafood products during storage

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ABSTRACT

Gwamegi (half-dried Pacific herring or Pacific saury) and semi-dried squid are not subject to any heat treatment during processing, and can become contaminated with norovirus (NoV). This study investigated the effects of gamma irradiation (0, 3, 5, 7, and 10 kGy) on the inactivation of murine norovirus-1 (MNV-1) and the survival of MNV-1 in *Gwamegi* and semi-dried squid samples following their irradiation and storage at 10 °C for 7 days. The overall reductions of MNV-1 in *Gwamegi* were 0.66, 0.88, 1.31, and 1.66 $\log_{10}$ PFU/ml by 3, 5, 7, and 10 kGy irradiation, respectively. The overall reductions of MNV-1 in semi-dried squid were 0.59, 0.88, 1.36, and 1.81 $\log_{10}$ PFU/ml by 3, 5, 7, and 10 kGy irradiation, respectively. There was an approximate 1 $\log_{10}$ reduction in viral load for all irradiated samples stored at 10 °C for 7 days at doses of 3, 5, 7, and 10 kGy including non-irradiated samples. Our findings suggest that gamma irradiation using a dose $\geq$7 kGy could be effective in reducing MNV-1 titers by more than 1 $\log_{10}$ PFU/mL (>90%) in half-dried seafood products, without any concomitant changes in color and sensory evaluation.

1. Introduction

The populations of many countries have used drying as a traditional method of food preservation (Jain & Pathare, 2007). In addition, fully drying fishery products can inhibit the growth of microorganisms (Doe, 1998). However, there is an increasing preference for half-dried food products because they have better flavor and are more tender than their fully dried counterparts even though fully dried food products can be stored for much longer periods than half-dried food (Gou, Choi, & Ahn, 2012). Thus, many aquatic species are consumed as half-dried products in Korea, including shellfish and mollusks. With the increase in consumption of these half-dried products, better controls over microbial growth are required because half-dried food products contain more moisture than those that are fully dried (Lee, Oh, & Choi, 2008a), resulting in increased microbial activity (Baert, Debevere, & Uyttendaele, 2009).

Squid (*Todarodes pacificus*) and *Gwamegi* which are made with Pacific saury (*Cololabis saira*) or Pacific herring (*Clupea pallasii*) are traditionally consumed as half-dried products in Korea. They are dried under natural conditions, until its moisture content drops to approximately 35–40% during winter (Lee, Jo, Cha, Kim, & Byun, 2002, Lee, Oh, Jeong, & Choi, 2008b). They are also not subject to any sterilizing or heating procedures after drying and prior to its consumption (Choi, Park, & Shin, 2012). In addition, there are no specific safety regulations of half-dried fishery products in place regarding the production and distribution in Korea, at present (Kim, Lee, Cho, Yook, & Byun, 2000; Lee et al., 2002); therefore, there is a need to monitor and control pathogenic microorganisms that might have been introduced during manufacturing and distribution. Thus, the freshness of semi-dried squid and *Gwamegi* must be maintained during its distribution across Korea, and efforts have been made to extend the shelf life of this half-dried seafood product.

There are a number of pathogenic microorganisms that can infect and survive in the aforementioned fish species. Of these microbes, noroviruses (NoVs) are a major concern, as they are a common cause of acute gastroenteritis in humans (Anonymous, 2015). NoVs are highly infectious and there is no specific or effective therapy against NoV infection (Anonymous, 2015). To protect against NoV infection, food products derived from marine environments should be monitored for NoV contamination (Rzezutka & Cook, 2004). NoV outbreaks have occurred numerous times as a

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result of contaminated seaweed and shellfish that were collected from contaminated seawater (Anonymous, 2015). Half-dried seafood products should also be monitored for the presence of NoVs and subjected to pre-emptive decontamination procedures during processing.

In the 1950s, US, Canada, and EU began using irradiation to extend the shelf life of certain foods, and to eliminate microorganisms during food processing. It has been found that food exposed to a dose of up to 10 kGy is safe for toxicology, nutrition and microbiology by the joint FAO/WHO/IAEA Expert Committee (Codex alimentarius commission, 1999). Since then, many studies have been conducted to examine the effects of gamma radiation upon the elimination of pathogenic microorganisms (Bidawid, Farber, & Sattar, 2000; Choi et al., 2010; Kim et al., 2010). International organizations such as the WHO, FAO, IAEA, and CODEX have established regulations for food irradiation, and this technology has been widely adopted in commercial settings. A maximum dose of 10 kGy of gamma radiation is acceptable for eliminating pathogenic microorganisms from general food products. A dose of up to 7 kGy is considered acceptable for fish and other marine products.

Although much research has been carried out to the effects of gamma radiation on microbes, its effects on human NoV are not widely known. Until recently, it has not been possible to maintain NoVs in cell culture; however, murine norovirus-1 (MNV-1) can now be cultured (Duizer et al., 2004). Results from recent studies have shown that MNV-1, a member of the *Caliciviridae* (Atmar & Esters, 2006), is a suitable surrogate model for human NoV (Wobus, Thackray, & Virgin, 2006). In the current study, we investigated the effects of gamma irradiation at 0–10 kGy on the inactivation of MNV-1 as a human NoV surrogate in *Gwamegi* and semi-dried squid. In addition, we assessed MNV-1 survival inoculated in *Gwamegi* and semi-dried squid under cold storage conditions (10 °C) during a week following irradiation. The effects of gamma irradiation on the physicochemical (color and lipid peroxidation) and sensorial quality in *Gwamegi* and semi-dried squid were evaluated during the storage periods.

2. Materials and methods

2.1. Virus and cell culture

Murine norovirus strain MNV-1 was supplied from Dr. Skip Virgin, Washington University. MNV-1 was maintained in the murine macrophage RAW 264.7 cell line, which we purchased from the American Type Culture Collection (Rockville, MD, USA). Cultivation of cells was performed in accordance with the direction of American Type Culture Collection (ATCC) TIB-71™. We prepared virus suspensions as described previously, with some modifications (D'Souza & Su, 2010). RAW 264.7 cells that were 80% confluent were transferred to 150-cm^2 tissue culture flasks. Growth medium was aspirated and cells were washed with phosphate-buffered saline (PBS, pH 7.4). A 1 mL aliquot of MNV-1 was added to the cells, and cultures were incubated at 37 °C/5% CO_2 for 1 h to allow the virus to adsorb. Maintenance medium (DMEM supplemented with 2% FBS) was then added, and cultures were incubated at 37 °C/5% CO_2 until cytopathic effects were observed in >90% of cells. The virus-infected flasks were frozen and thawed three times (−80 °C and 37 °C) to lyse cells and release virus particles. Lysates were dispensed into 50 mL centrifuge tubes and centrifuged (1500 × *g*, 10 min) to clarify supernatants. The virus-containing supernatants were stored at −80 °C until required.

2.2. Sample preparation and inoculation

Gwamegi and semi-dried squids were purchased from a local supermarket in Anseong (Korea). Individual *Gwamegi* and semi-dried squids were cut into pieces (3.0 × 3.0 × 0.5 cm) that approximately weighed 5 g using a sterile stainless steel knife, with each piece placed into sterile petri dishes (50 mm in diameter and 10 mm in depth). A 200 μL aliquot of MNV-1 (6 $\log_{10}$ PFU/mL) was inoculated on the surface of each sample. To allow the virus to attach to the surfaces of the food samples, the samples were placed on a clean bench for 1 h at room temperature. The samples were then vacuum packed (Rollpack vacuum roll, Rollpack Inc., Korea) like the normal type of packaging in the markets using a Food Keeper VP-9900 vacuum packaging system (Rollpack Inc., Korea). For viral suspension test, the experiment was performed as previously described with slight modifications (Jean, Morales-Rayas, Anoman, & Lamhoujeb, 2011). A 1 mL of MNV-1 suspension which was prepared as described in Section 2.1 (6 $\log_{10}$ PFU/mL) was put into sterile 50 ml conical tube.

2.3. Irradiation source

All the samples were stored in an icebox and delivered to the Korea Atomic Energy Research Institute in Jeongeup, Korea. Samples were irradiated using a Cobalt-60 (Point Source, ACEL, IR-79, MDS Nordion, Ontario, Canada) course, at four different doses (3, 5, 7, and 10 kGy); control samples were not irradiated. The strength of the radiation source was approximately 11.1 PBq at a dose rate of 10 kGy/h. Dosimetry was performed using alanine dosimeters (5 mm diameter; Bruker Instruments, Rheinstetten, Germany), with the free radical signal measured using a Bruker EMS 104 EPR Analyzer. Dosimeters were calibrated against an international standard developed by the International Atomic Energy Agency (Vienna, Austria).

2.4. Storage

Both *Gwamegi* and semi-dried squid which were irradiated at four different doses (3, 5, 7, and 10 kGy) and were not irradiated were stored at 10 °C for a week. The storage experiments were performed on 0, 3rd, 5th, and 7th day. All samples were assessed the survival of MNV-1, surface color, 2-Thiobarbituric acid reactive substances (TBARS) analysis, and sensory evaluation.

2.5. Sample processing for virus recovery

Virus recovery from *Gwamegi* and semi-dried squid samples was performed as previously described, with slight modifications (Son et al., 2014). Following the gamma irradiation, each virus-contaminated sample (5g) was placed into a 50 mL centrifuge tube and 5 mL of PBS was added. Samples were subjected to thorough vortexing and shaking (300 rpm, 1 h), followed by centrifugation (10,000 × *g*, 1 h, 4 °C). Supernatants were sequentially filtered using sterile 5, 1.2, 0.8, and 0.45 μm filters. Each eluted viral suspension was then 10-fold serially diluted in DMEM. The virus infection titers were analyzed with a plaque assay.

2.6. Virus titration

Plaque assays were performed as previously described, with some modifications (Bidawid, Malik, Adegbunrin, Sattar, & Farber, 2003). RAW 264.7 cells were grown in 12-well plates (4 × 10^5 cells/well) and incubated cultures at 37 °C/5% CO_2 until they were 90% confluent. Each cell was inoculated with 0.2 mL of diluted MNV-1 isolated from seafood products and allowed to adsorb for 1 h at 37 °C. Cells were overlaid with 1 mL of 2 × type II agarose mixed with 2 × DMEM and left at room temperature until the agarose mixture solidified. Cultures were then