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Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi

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ABSTRACT

Optimally ripened commercial cabbage kimchi is considered the main cause of enteric norovirus (NoV) outbreaks in Korea. This study investigated the effect of 1–10 kGy gamma radiation on the inactivation of murine norovirus-1 (MNV-1; initial inoculum of 5–6 $\log_{10}$ PFU/ml), used as a human NoV surrogate, in kimchi. The effects of gamma radiation on the pH and acidity were also examined to address the index of quality and fermentation, respectively. Titers of MNV-1 significantly reduced ($p < 0.05$) in kimchi subjected to increasing gamma radiation doses: MNV-1 titers in kimchi after 1, 3, 5, 7, and 10 kGy were 4.82 (0.34-$\log_{10}$ reduction), 4.45 (0.71-$\log_{10}$ reduction), 4.18 (0.98-$\log_{10}$ reduction), 3.71 (1.45-$\log_{10}$ reduction), and 3.40 (1.76-$\log_{10}$ reduction) $\log_{10}$ PFU/ml, respectively. However, the values of pH (4.5–4.6) and acidity (0.6–0.7%) were not significantly different between non-irradiated and irradiated kimchi ($p > 0.05$). The D-value (1-log reduction) for MNV-1 in kimchi, calculated using first-order kinetics, was 5.75 kGy ($R^2 = 0.98$, RMSE = 0.10). Therefore, this study suggests that the use of ≥ 5.75 kGy gamma radiation in the kimchi manufacturing industry could be very effective in reducing NoV contamination by > 90% (1 log), without causing changes in quality and fermentation.

1. Introduction

Kimchi is the most consumed traditional fermented food in Korea. It is fermented by probiotic lactic acid bacteria and is made of fresh vegetables, such as cabbage, radish, scallion, and cucumber, soaked in bamboo salt and mixed with spices and seasoning. It can be fermented for a few days or a few weeks, depending on the storage temperature. According to the report of the Codex Alimentarius Commission (Codex, 2001), kimchi, as a preserved food, can contribute with its high nutritional value to most Koreans throughout the year, as it is low in calories (18 kcal/100 g) and rich in vitamins (vitamin B complex, vitamin C, and β-carotene), minerals (Na, Ca, K, Fe, and P), and dietary fiber (24% on a dry basis). Moreover, kimchi has been known as a healthy functional food due to its anticonstipation, anticancer, antioxidation, antiatherosclerotic, antidiabetic, and antiobesity effects, and overall immune promotion (Park, Jeong, Lee, & Daily, 2014). Given the health benefits of kimchi and the increasing number of people having meals at institutional foodservice places, such as schools, hospitals, and companies, and at restaurants, the consumption of kimchi has increased (Seo, 2014). This means that, currently, kimchi is generally produced on a commercial and large scale. The market size of the kimchi industry in Korea has steadily increased from $2.1 billion to $2.5 billion in 2013 (Seo, 2014). In 2001, a CODEX standard for cabbage kimchi, one of the 200 different types of kimchi, was established (Codex, 2001). Furthermore, cabbage kimchi was selected as one of the healthiest foods by a US health magazine, in 2006. Nevertheless, if its safety, quality, and nutrition are not assured, kimchi cannot play a role as an international and global food. Hence, the application of the HACCP system at commercial cabbage kimchi manufacturing places has been made mandatory by the Korean MFDS (Ministry of Food and Drug Safety) since 2006. Despite HACCP implementation, commercial cabbage kimchi has been recently regarded as a potential cause of human norovirus (NoV) outbreaks in Korea, as the virus has been occasionally detected in commercial kimchi. Commercial cabbage kimchi contaminated with NoV was the cause of the acute gastroenteritis outbreaks that affected twenty-two students in three junior high schools in 2013, in the Jeonju province, in Korea. In 2011, five consecutive gastroenteritis outbreaks were reported at schools in the Gyeonggi-do province, in Korea (Lee, Kim, Cho, & Lee, 2012), in which norovirus-contaminated commercial cabbage kimchi was the cause (Lee et al., 2012). NoV GI.4 was the NoV dominant genotype detected in cabbage kimchi samples (Park et al., 2015).

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NoV is considered one of the main causative agents of viral foodborne illnesses. This non-enveloped icosahedral virus, with a single-stranded and positive-sense RNA genome, belongs to the *Norovirus* genus within the *Caliciviridae* family (Noda, Fukuda, & Nishio, 2008; Siebenga et al., 2009). NoV is the most serious viral agent of acute gastroenteritis among enteric viruses (Hewitt, Rivera-Aban, & Greening, 2009). The common signs and symptoms of acute gastroenteritis are nausea, vomiting, diarrhea, and abdominal pain (Atmar et al., 2008). Transmission of NoV usually occurs through the fecal-oral route, by the ingestion of contaminated food and water, through the direct contact with infected individuals, or through aerosols produced by an infected individual (Duizer et al., 2004). NoV has been shown to successfully replicate in human B cells (Jones et al., 2014; Jones et al., 2015), although the level of viral replication is modest ($< 2 \log_{10}$) and the use of the B cell model to study natural NoV infection is inconsistent (Jones et al., 2015). More recently, it has been shown that NoV can also successfully replicate in stem cell-derived human enteroides; nevertheless, some NoV strains require bile for replication of (Ettayebi et al., 2016). Additionally, the lack of suitable histoblood group antigen expression in intestinal cells can restrict virus replication, and infectivity is abrogated by inactivation methods, such as irradiation and heating, and serum neutralization (Ettayebi et al., 2016). Therefore, currently, these two model systems are not accessible or feasible. Since NoV cannot grow in conventional cell cultures, hindering studies on control measures, a murine norovirus (MNV-1), which is cultured in a murine dendritic cell line (RAW 264.7), has been widely used as model. MNV-1 has characteristics similar to NoV regarding genetic organization and route of transmission, i.e. fecal-oral route (Karst, Wobus, Lay, Davidson, & Virgin, 2003; Kingsley, Holliman, Calci, Chen, & Flick, 2007; Wobus et al., 2004).

Radiation technology has been used in developed countries, such as the US and Canada and in the EU, since the 1950s, to extend the shelf-life of certain foods and to eliminate microorganisms during food processing. Irradiation with gamma rays is the most widely accepted form of radiation sterilization and is generally used when materials cannot be heated to high temperatures. Irradiation of food is generally considered safe and poses no significant risk to human health (Bari et al., 2005; Khattak et al., 2005; Sommers & Rajkowski, 2011). Exposure of food to a dose ≤ 10 kGy was declared safe in terms of toxicology, nutrition, and microbiology by the joint FAO/WHO/IAEA Expert Committee (Codex, 1999). Since then, several studies have been conducted to examine the effects of gamma radiation on the elimination of pathogenic microorganisms (Bidawid, Farber, & Sattar, 2000; Choi et al., 2010; Kim et al., 2010). International organizations such as 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 to eliminate pathogenic microorganisms from general food products (Bidawid et al., 2000; Park, Kang, & Ha, 2016; Song et al., 2012). Gamma radiation can also be applied to improve the quality and shelf-life and to control the ripening process of kimchi products (Kim, Yook, & Byun, 2004).

The efficacy of gamma radiation against foodborne NoV has not yet been reported in kimchi; therefore, there are no Korean or international regulations regarding gamma irradiation of diverse kimchi products, including cabbage kimchi, for the control of NoV. The objective of the present study was to investigate the effectiveness of irradiation with gamma rays (0–10 kGy) in the control of NoV infectivity. We used MNV-1 as a NoV surrogate to experimentally contaminate commercial cabbage kimchi, which is the most popular and common type of kimchi in Korea.

2. Materials and methods

2.1. Virus cell culture

MNV-1 (a surrogate for human NoV) was maintained in murine RAW 264.7 cells. Cells were cultured in Dulbecco's minimum essential medium (DMEM; Sigma, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 44 mM sodium bicarbonate (Sigma, USA), and 1% antibiotic-antimycotic (Penicillin Streptomycin; Gibco, USA), in 75 cm^2 culture flasks, at 37 °C, in a humidified incubator containing 5% CO_2. Cells were subcultured every 2–3 d.

2.2. Preparation of viral suspension

When RAW 264.7 cell monolayers reached 90% confluency in 150 cm^2 tissue culture flasks, the growth medium was aspirated, and the monolayers were washed with phosphate-buffered saline (PBS, pH 7.4; Oxoid, Basingstoke, Hampshire, England). A 200-μl aliquot of the MNV-1 inoculum was added to each flask, and the flasks were incubated for 30 min to allow virus adsorption. Then, 15 ml of the maintenance medium (DMEM supplemented with 2% FBS, 44 mM sodium bicarbonate, and 1% antibiotic-antimycotic) were added, and the cultures were incubated at 37 °C, in 5% CO_2 atmosphere, for 3 d. If > 90% cytopathic effects were observed, virus-infected cultures were subjected to three freeze-thaw cycles (− 70 °C and 37 °C, respectively) to lyse cells and release virus particles. Lysates were centrifuged at 1500 × *g* for 10 min to remove cell debris, and the supernatants were harvested. Viruses were stored at − 70 °C until further use.

2.3. Preparation of cabbage kimchi and virus inoculation

One pack of cabbage kimchi (1 kg) manufactured by Ourhome company (Chungbuk, Korea) was purchased from a local market (Ansung, Korea). Ten grams of kimchi (5 g of cabbage kimchi and 5 g of cabbage kimchi juice) were weighed using a sterile stainless steel knife and spoon. A 500-μl aliquot of MNV-1 (5–6 $\log_{10}$ PFU/ml) was inoculated into the cabbage leaf, stem surfaces, and kimchi juice. The samples were then vacuum-packed (Rollpack vacuum roll, Rollpack Inc., Korea), similarly to the normal packaging found in markets, using a Food Keeper VP-900 vacuum packaging system (Rollpack Inc., Korea).

2.4. Gamma radiation

Kimchi was stored in an icebox and delivered to the Korea Atomic Energy Research Institute, in Jeongeup, Korea. Samples were exposed to 0, 1, 3, 5, 7, or 10 kGy of gamma rays, using a cobalt-60 gamma irradiator (Point Source, ACEL, IR-79, MDS Nordion, Ontario, Canada), at the Korea Atomic Energy Research Institute (Jeongeup, Korea). The source strength was approximately 11.1 PBq, at a dose rate of 10 kGy/h. Dosimetry was performed using 5-mm diameter alanine dosimeters (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).

2.5. Sample processing for virus recovery and virus titration

MNV-1 recovery from the samples was performed as described previously, with slight modifications (Son et al., 2014). Briefly, following gamma radiation treatment, each contaminated sample (10 g) was placed into a 50-ml centrifuge tube, and 10 ml of PBS were added. The 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-mm filters. Each eluted viral suspension was then 10-fold serially diluted in DMEM. The virus infection titers were determined using a plaque assay.

MNV-1 titration was performed as described previously, with minor modifications as suggested by Wobus et al. (2004) and Bidawid et al. (2000). Briefly, confluent RAW 264.7 cells were seeded into each well of a 12-well plate and incubated at 37 °C, with 5% CO_2, for 36 h, until

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