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Synergistic effect of the combined treatment with gamma irradiation and sodium dichloroisocyanurate to control gray mold (*Botrytis cinerea*) on paprika

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HIGHLIGHTS

- Paprikas were treated with irradiation and NaDCC to control gray mold.
- We confirmed that the combined treatment was synergistically affected.
- The treatment can contribute to a reduction of postharvest losses caused by fungi.
- This combined treatment can also reduce the doses of irradiation.

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ABSTRACT

Gray mold (*Botrytis cinerea*) is one of the most major fungal pathogens in paprika. Generally, gamma irradiation over 1 kGy is effective for the control of fungal pathogens; however, a significant change in fruit quality (physical properties) on paprika was shown from gamma irradiation at over 0.6 kGy ($p < 0.05$). Therefore, in this study, the synergistic disinfection effect of the combined treatment with gamma irradiation and sodium dichloroisocyanurate (NaDCC) was investigated to reduce the gamma irradiation dose. In an artificial inoculation experiment of *B. cinerea* isolated from naturally-infected postharvest paprika, fungal symptoms were observed in the stem and exocarp of paprika after conidial inoculation. From the sensitivity of gamma irradiation and NaDCC, *B. cinerea* conidia were fully inactivated by 4 kGy of gamma irradiation (D_{10} value 0.99 kGy), and were fully inactivated by 50 ppm NaDCC treatment. The fungal symptoms were not detected by the dose-dependent gamma irradiation (> 4 kGy) and NaDCC (> 50 ppm). As a result of the combined treatment of gamma irradiation and NaDCC, the D_{10} value was significantly reduced by 1.06, 0.88, 0.77, and 0.58 kGy ($p < 0.05$). Moreover, fungal symptoms were more significantly reduced in combined treatment groups (gamma irradiation and NaDCC) than single treatment groups (gamma irradiation or NaDCC). These results suggest that combined treatment with irradiation and NaDCC treatment can be applied to preserve quality of postharvest paprika or other fruits.

1. Introduction

Paprika (spice red pepper; *Capsicum annuum* L.) is one of the most economically important fruit crops. It is an excellent vitamin source which has been confirmed by various epidemiological studies (Gey and Puska, 1989; Gerster, 1991). In spite of this economic importance, the productivity decreases and quality losses occur during the preharvest and postharvest management of this fruit crop because of a number of diseases. The gray mold caused by *Botrytis cinerea* develops mainly on fruits, leaves, or flowers (Dik and Elad, 1999) in a greenhouse, causing a decrease in the productivity of paprika throughout the world, and is usually managed by the frequent use of prophylactic fungicides (Bulger et al., 1987; de Visser, 1996; Raposo et al., 1996) in fruit crops, including pepper crops during preharvest management. Furthermore, *B. cinerea* causes an important postharvest decay of pepper (Pernezny et al., 2003), and disinfection technologies of this pathogen are needed for a postharvest management strategy.

Most postharvest management technologies have applied chemical fumigation for the preservation of fruit quality from fungi mediated diseases, but alternative methods are needed because of

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growing public concerns over the human health and environmental risks (Spotts and Cervantes, 1986). Therefore, many researchers have focused on eco-friendly postharvest technologies that can contribute to replacing the use of chemical fumigation techniques for crop quality preservation. Alternative treatments have been reported: heat treatments (Lurie, 1998), photochemical treatments (Baka et al., 1999), pulsed light treatments (Gómez-López et al., 2005b), microwave drying (Orsat et al., 2006), UV treatments (Vicente et al., 2005), and ozone treatments (Bialka and Demirci, 2007). Although these alternative treatments show promise, individual treatments are not effective for fungicides. Therefore, it is necessary to develop a technology that combines other disinfection treatments for synergistic effects (Conway et al., 2005).

Although fresh fruits and vegetables can be irradiated at a gamma ray dose of up to 1 kGy (US FDA, 2004), this is not possible to fully control postharvest fungi (Blank and Corrigan, 1995). Moreover, the radiation dose required to control fungi has a negative effect on the skin color and texture of stored fruits and vegetables (Beraha et al., 1960; Tiryaki et al., 1994). Therefore, it is important to reduce the irradiation dose for an inhibition of postharvest photogenes through a combined treatment. One promising treatment is the use of gamma irradiation with a combination of other treatments. Consequently, this combined treatment can contribute to a reduction of postharvest losses caused by fungi and reduce the use or doses of fungicides for disease control (Cia et al., 2007).

Chlorination is applicable to specific fruits and vegetables, including paprika, using a postharvest process with flumes, water dump tanks, and spray washers. This postharvest treatment was applied to various fruits and vegetables such as tomatoes (Goodin, 1977; Showalter, 1993), citrus (Hough and Kellerman, 1971), apples (Hendrix, 1991), pears (Sanderson and Spotts, 1995), and peppers (Sherman and Allen, 1983).

In this study, the effect of gamma irradiation on the physical property of postharvest paprika was evaluated using a texture analyzer. The sensitivity of gamma irradiation and dichloroisocyanurate (NaDCC) was evaluated through *in vivo* and *in vitro* tests. The objective of the present study is to evaluate the combination effect of gamma irradiation and NaDCC for disease control and shelf-life of the paprika.

2. Materials and methods

2.1. Preparation of paprika and fungus

Paprika was purchased from an agricultural expert company (Rosepia Co., Ltd., Jeollabuk-do, Korea). The paprika was selected for a uniform size and freedom from defects, surface sterilized with 2% sodium hypochlorite for 1 min, rinsed in sterile distilled water (DW), and dried on a clean bench.

The fungus was isolated from a naturally-infected paprika stem. For a pure culture, isolated individual conidia were transferred to potato dextrose agar (PDA, Difco Laboratories, Detroit, MI). All cultures were grown on PDA at 25 °C for 14 days. Cultures were transferred to a fresh PDA every 30 days. For fungal identification, the genomic DNA was isolated from the fungus using a DNA extraction kit (Bioneer, Korea) according to the manufacturer's protocols. The extracted DNA was purified using a QIAquick® DNA purification kit (Qiagen, Valencia, CA, USA). PCR amplification of 18s rRNA genes was carried out in 50 μL PCR reactors using the primers ITS1 and ITS4 (White et al., 1990). PCR products were purified using the QIAquick®Gel Extraction Kit (Qiagen), and gene sequencing and a blast search were requested from a commercial analysis service (Solgent Inc., Daejeon, Korea).

2.2. Preparation of fungal conidia

For harvesting the conidia, about 10 mL DW was added to a culture dish and the conidia were gently harvested by filtration through four sterile layers of gauze. The conidial suspensions were collected in sterile screw-cap test tubes (16×100 mm^2) containing 15 mL of sterile distilled water and filtered twice using sterile Pasteur pipettes (4.62 mm) packed with glass wool. The procedure was used to remove mycelial fragments and conidial clumps (Saleh et al., 1988). The concentration of conidia was measured using a hemacytometer (Warner-Lambert Technologies Inc., Buffalo, N.Y.). The final conidial concentration was adjusted to 10^5 conidia mL^{-1} with DW for further study (Slade et al., 1987).

2.3. Gamma irradiation and NaDCC treatment

The samples were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada) with various absorbed doses (from 0.2 to 4 kGy). Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). For chlorination, the samples were immersed in sodium dichloroisocyanurate (NaDCC; Sigma-Aldrich, Poole, UK) at various concentrations (from 5 to 50 ppm). Following treatment, the suspensions were serially diluted in DW and plated on PDA. Incubation was carried out at 25 °C for 5 days. Survival curves were constructed by plotting the survivor CFU/mL versus the actual radiation doses and NaDCC concentrations. The curves were fitted by linear regression, and the radiation sensitivity was expressed in terms of the D_{10} values. A D_{10} value is defined as the dose required for reducing the given population by 90% of its initial value. The D_{10} value was determined from the reciprocal of the slope for the straight-line portion of the survival curve (Ley, 1983).

2.4. Artificial inoculation on paprika

Pre-sterilized paprika fruits were used in an artificial inoculation test, and was wounded to a 1.5 mm thickness by puncturing them with the point of a sterile 0.5 mm diameter needle. Each wound site was artificially inoculated with 10 μL of suspension containing 10^5 conidia mL^{-1} on the paprika. To protect secondary contamination, the inoculated paprika was transferred into a sterilized oxygen-impermeable plastic container, and was kept at 25 °C and a relative humidity of $>90\%$ for 1 week.

2.5. Physical properties of irradiated paprika

Following gamma irradiation, the physical properties were monitored from a tissue of rectangular shaped irradiated and control paprika. Tissues (3 cm × 1 cm) were trimmed from the exocarp to endocarp tissue with razor blades. Thirty tissues from 10 samples were tested using a textural measurement instrument (model TA-XT2i, stable Micro System, Surrey, UK) equipped with a 5 kg mechanical load cell, and were compressed 3 times per sample with a 2-mm-diameter puncture probe. Data are presented as the maximum force of hardness and fracturability recorded during tissue compression.

2.6. Statistical analysis

All experiments were carried out in triplicate with three observations. A one-way analysis of variance was performed using the SPSS software system, and Duncan's multiple range tests were used to compare the differences among the mean values.