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Use of Ionizing Radiation as a Phytosanitary Treatment for Postharvest Disease Control

Rae-Dong Jeong*

Research Division for Biotechnology, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea

Abstract - Postharvest diseases cause considerable losses to harvested fruits and vegetables worldwide. Fresh produce suspected of harboring postharvest disease must be treated to control any pathogens present. Although there are various treatments to control postharvest losses by pathogens, the current community is eager to take safer and more eco-friendly alternatives to help with human health and reduce environmental risks. Ionizing irradiation is a promising phytosanitary treatment that has a significant potential to control postharvest diseases in use worldwide. Although almost 19000 metric tons of sweet potatoes and various fruits are irradiated each year in six countries to control postharvest disease, irradiation continues to be a debate, with slow acceptance by industries. Irradiation alone is not effective as a fungicide, and an over dose affects the physical properties of irradiated products. A combination of irradiation with other treatments such as heating, biocontrol agents, chlorination, and nano Ag particles is to enhance their effectiveness. Challenges to the use of phytosanitary irradiation are an avoidance of irradiated postharvest and cost of the irradiation facilities, and thus consumers still need to be educated on the principles and benefits of irradiation and prepare an optimum economy of scale for commercial use. In this review, we evaluated the current phytosanitary irradiation, and combination with various other treatments to minimize the postharvest losses.

Key words : Postharvest diseases, Ionizing irradiation, Phytosanitary irradiation

INTRODUCTION

A phytosanitary measure is used to prevent the introduction or spread of regulated pests while permitting trade. This legal treatment affects mortality, preventing the successful development, sterility, and inactivation in the quarantine pests. By increasing all traded agricultural products linked to fresh produce, effective phytosanitary treatment is desperately needed for preventing the dissemination of alien invasive pests (IPPC 2003).

The Food and Agriculture Organization of the United Nations (FAO) has estimated that approximately 25% of potential profits in all global food production are lost after harvest owing to diseases caused by fungi and/or bacteria, physical injuries from insects, mechanical force, chemical heating or freezing, and non-disease disorders resulting from storage conditions. Postharvest losses are estimated to range 20 to 40% in developing countries and 10 to 15% in developed countries per year by postharvest diseases, depending on the crop despite the use of modern storage facilities (Braghini *et al*. 2009). Most crop losses by pathogens caused by fungi and bacteria (Table 1).

Postharvest losses are 5 to 10% when postharvest fungicides are treated; whereas without fungicides, they will reach up to 50% in years (Margosan *et al*. 1997). There are some

* Corresponding author: Rae-Dong Jeong, Tel. +82-63-570-3193, Fax. +82-63-570-3195, E-mail. jraed2@kaeri.re.kr

techniques such as extreme temperatures and chemical fumigants to control diseases as phytosanitary treatments. A list of the major commercially used phytosanitary treatment is provided in Table 2. Among them, chemical fumigants such as methyl bromide, fludioxonil, and azoxystrobin are dominantly used to control diseases or decay in products. However, the use of chemical fumigants for postharvest is prohibited due to fumigant regulatory issues, public demands for environmental and health issues, and occurring resistant pathogens to the application of chemicals in products (Hallman 2011).

The Montreal Protocol (UNEP 2000) was released for developing countries to stop all uses of methyl bromide by 2015, except for plant quarantine and critical emergencies. It is necessary to look into alternatives to control postharvest diseases to substitute the chemical fumigants. Although several methods, such as cold storage, heating, and modified atmosphere storage, have been tested, none of them have shown the effectiveness of fungicides. Until the late 1920s, phytosanitary treatments used fumigation or nonsynthetic pesticide application. An outstanding alternative method is the use of irradiation recommended by FAO for national phytosanitary programs (Conway *et al*. 2005). The publication in International Standard for Phytosanitary Measure no. 18 "Guidelines for the use of irradiation as a phytosanitary measure" was released for the recommendation of radiation technology as a means to international trade in horticultural products, and is open method to incorporate additional irradiation uses for crop protection (IPPC 2003).

The goal of this review is to describe the current use of irradiation as a phytosanitary treatment to control postharvest diseases in horticultural products and suggest approaches to optimize its potential. In addition, the present use of radiation technology as well as the synergistic disinfection effects of combined treatment with eco-friendly agents and radiation for further applications is also discussed.

MAIN SUBJECT

The irradiation process

Radiation is generally referred to as energy emitted from a source. Ionizing radiation is radiation with sufficient energy that during an interaction with an atom, it can break the chemical bonds owing to electromagnetic radiance or high-energy particles. There are several forms of electromagnetic radiation such as heat waves, radio waves, infrared light, visible light, ultraviolet light, X-rays, and gamma rays depending on the frequency and wave length. Currently, only three sources of ionizing radiation, gamma-rays, e-beams, and X-rays, are commercially used for phytosanitary purposes. Gamma rays given off by the isotopes cobalt-60 (1.17 and 1.33 MeV) and cesium-137 (0.66 MeV) are legally used for phytosanitary irradiation. Cobalt-60 is generated from standard nonradioactive cobalt and has a half-life of 5.27 y, whereas cesium-137 is a product of the fission of uranium and plutonium and has a half-life of 30.07 y. Although both are commercially accepted, cobalt-60 is mainly used at the commercial scale due to its half-life and soluble availability in water. Electrons and X-rays are produced by machine sources of radiation. An electron beam (e-beam) will produce X-rays, and about 14% of the energy from an e-beam is converted into X-rays.

The unit of Irradiation dose is the gray (Gy), which is the energy absorbed in J kg^{-1} of material (the discontinued unit is the rad, which equals 0.01 Gy). The absorbed dose is normally referred to as the amount of ionizing radiation energy treated on the product. There are three types of dose categories in ionizing radiation: (1) low dose of up to 1 kGy for the delay of ripening and insect disinfection, (2) medium dose of 1 to 10 kGy for non-spore forming pathogens and reduction of spoilage microorganism, and (3) high dose of 10 to 50 kGy for the sterility of microorganisms.

Although gamma rays and e-beams or X-rays are produced by different sources, isotopes, or machine sources, they have the same mode of action. However, gamma rays are most commonly used in food irradiation because of their ability of penetration. They cause atoms and molecules to become ionized or excited, thereby producing free radicals, breaks chemical bonds, and damage molecules involved in the cell process (Fig. 1). In addition, different levels of dose requirement of irradiation have been applied for respective purpose. A low dose of irradiation inhibits sprouting in tubers, bulbs, and root vegetables (potato, onions), and delays the ripening of tropical fruits, whereas a high dose of irradiation usually results in an extension of shelf-life and inactivation of pathogenic microorganisms (Table 3).

The sensitivity of an organism is correlated with its complexity. Thus, viruses show the highest resistance to inacti-

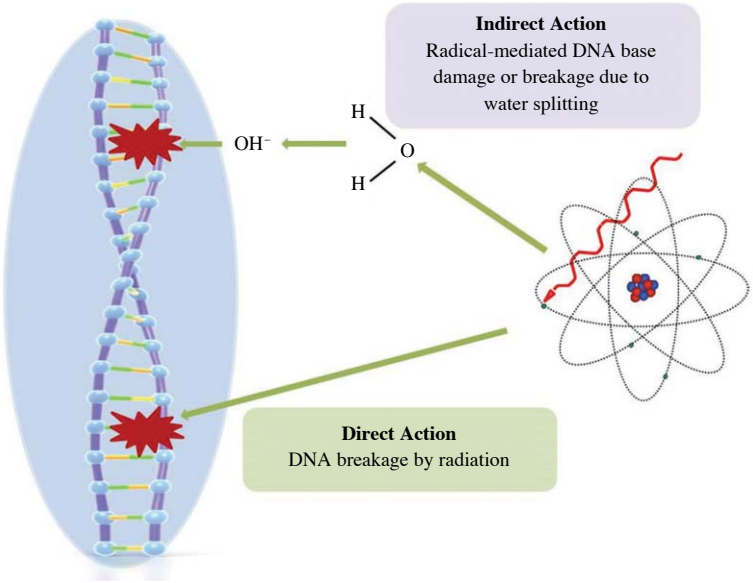

Fig. 1. Biological effects of ionizing radiation. In the first scenario, radiation may impact the DNA directly, causing ionization of the atoms in the DNA molecules. This could be visualized as a "direct hit" by the radiation on the DNA, and thus is an uncommon occurrence due to the small size of the target. In the second scenario, the radiation interacts with non-critical target atoms or molecules, usually water. This results in the production of free radicals, which are atoms or molecules that have an unpaired electron and thus are highly reactive. These free radicals can then attack critical targets such as the DNA. Damage from indirect action is much more common than damage from direct action.

vation by irradiation, whereas insects and parasites are the most sensitive. Spores and cysts are resistant to irradiation owing to their little DNA. The D_{10} value is used as the killing effect of irradiation on microbes. One D_{10} value is the dose required to inactivate 90% of a population; for example, it takes 0.23 kGy to kill 90% of *Penicillium expansum*, and thus the D_{10} value of *P. expansum* is 0.23 kGy (Kim *et al.* 2010). The D_{10} values are different for each microbial pathogen due to their biological characteristics (Table 4). One of the most different things is that an X-ray heads in the same direction as the e-beam, while a gamma ray emits in all directions uniformly. An E-beam or X-ray can penetrate a few centimeters, while a gamma ray passes through thick walls. Due to the deeper penetration, a gamma ray is the mostly used method for a reduction of pathogens, indicating that irradiation is technically more effective than other pathogen treatments.

Applications of irradiation treatment on postharvest diseases

Irradiation is used as a pest control technology in over 40 countries, including the United States, Russia, Great Britiain, and Brazil. Losses caused by postharvest diseases are more severe than we expected because harvested fruits and vegetables are easily decayed by pathogens while passing from the field to the consumer despite the use of adequate storage and transportation facilities. Consumers are eager to want fruit or vegetables free of chemical residues. Moreover, the use of agrochemicals in commercial packinghouses causes a proliferation of resistant strains of the pathogens (Kinay *et al.* 2007). The use of gamma ray, electron beam and X-ray irradiation as a phytosanitary treatment has increased dramatically in recent years. This application of irradiation technology is critical due to uncertainties of the future and restrictions of methyl bromide under the Montreal Protocol, but still widely used for quarantine purposes. To date, even though gamma irradiation is a physical treatment that is effectively used for the control of postharvest diseases to avoid fungicidal effects, few studies have been conducted on handling postharvest diseases by irradiation (Conway *et al.* 2005). The effects of irradiation are dependent on fungal development, treatment dose, moisture condition, composition of products, and storage conditions (Aziz *et al.* 2006).

A study showed that D_{10} gamma values ranged from 0.236 to 0.416 kGy for *Penicillium* species. An e-beam is more effective than that of gamma sources because an e-beam can be directed at the pathogens, whereas the gamma sources

emit radiation in all directions. Gamma irradiation was used to inactivate the pathogens *Botrytis cinerea*, *Penicillium expansum*, *Rhizopus stolonifer* var. *stolonifer* and *Monilinia fructicola* in peach. The D_{10} values of each pathogens in peach were 0.14 kGy (*B. cinerea*), 0.23 kGy (*P. expansum*), 0.16 kGy (*R. stolonifer* var. *stolonifer*), and 0.16 kGy (*M. fructicola*) (Kim *et al*. 2010). Irradiation doses for the inhibition of fungal development are presented in Table 3 for several fungi. Gamma radiation is also used for postharvest disease incidence of pineapple fruits. Doses in the range of 50 to 250 Gy, in combination with storage at 11 to 13°C, could reduce the postharvest disease, *Penicillium purpurogenum* Stoll, in pineapples (Damayanti *et al.* 1992). Inhibition of spore germination is directly related to the strength of the radiation dose. *B. cinerea* and *P. expansum* are sensitive to radiation, while *Alternaria tenuissima* and *Stemphylium botryosum* are resistant to radiation. In addition, young mycelia are more resistant than mature mycelia. The lower doses of gamma radiation affect the increase in total proteins or total lipid production in *Fusarium solani* and *Penicillium notatum*, which suggest that proteins or lipids might play a role in protection against the harmful effects of radiation. Thus, identification of proteins or lipids involved in radiation of pathogens can show their roles as radio-protectors (Geweely and Nawar 2006). That is, ionizing radiation can stimulate the biosynthesis of compounds that can extend the storage life and induce fruit or vegetable resistance to pathogens (Palou *et al*. 2007).

In addition, low irradiation doses (under 1 kGy) stimulate fungal development both in vitro and in vivo in Ankara pears inoculated with *Penicillium expansum* (Tiryaki 1990). Moreover, 1 kGy of gamma irradiation increases the production of aflatoxin, but over 3 kGy suppresses development of fungal and mycotoxin. For the in vivo (in the host) test, higher doses of gamma radiation are required for pathogens compared to the in vitro (in the culture) test, suggesting that lots of factors are regulating the inactivation of pathogens by irradiation. However, there are some considerations that do not overlook the irradiated pathogens. When microorganisms are experimentally exposed to radiation, radiation-resistant pathogens occur, and in the case of fungi, the formation of mycotoxin increases. Thus, repeated products with ionizing energy cause a negative effect.

Another key factor to consider is the time interval between inoculation and irradiation. Kuhn *et al*. (1968) suggested that irradiation within 24 hr after inoculation show only 10% of the inoculation formed lesions. Consistent with this study, irradiation to 36, 48, or 60 hr after inoculation increased the disease severity to 60, 70, and 90%, respectively. However, in case of citrus *Penicillium*, 24 hr before the application of the antifungal treatment is the effective time for the control of infections (Palou *et al*. 2007).

Application of combined treatment for control of postharvest diseases in phytosanitary irradiation

Fresh fruits and vegetables can be irradiated at doses up to 1 kGy by a gamma ray (US FDA 2004), and thus it is tricky to fully control postharvest diseases. The radiation dose needed to completely control diseases has a negative effect on the skin color and texture of stored fruits and vegetables. Therefore, it is necessary to take advantage of combinations with other treatments such as heat, pulsed light, microwave drying, UV, ozone, environmental conditions, biocontrol agents, and eco-friendly chemicals, to increase the effectiveness and decrease the negative effect of the application (Fig. 2) (Vincente *et al*. 2005; Orsat *et al*. 2006). Consequently, the combined treatment can decrease postharvest losses caused by fungi and eventually reduce the use or doses of fungicides for control (Conway *et al*. 2004).

Synergistic effects of combined gamma radiation and heat treatment (38°C for 4 days) contributed 5- to 10-fold increases in the inactivation of spores of postharvest pathogens. Heat treatment may also affect the susceptibility of the host to

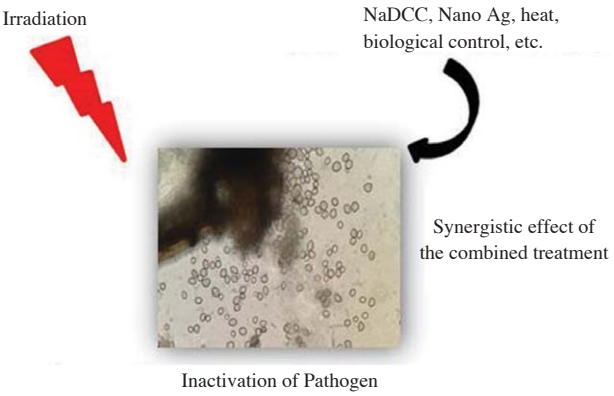

Fig. 2. Synergistic effect of the combined treatment on postharvest diseases. Combined treatment is to increase the effectiveness, to decrease the negative effect of application with lower dose compared to single application. Irradiation can be applied in combination with heat, NaDCC, Nano Ag, biological agent etc.

pathogens by an inhibitory substance in the peel. The combination of hot water and gamma irradiation showed a synergistical reduction of fungal development, resulting in 1.7% and 10.0% infections by *B. cinerea* and *R. stolonifer*, respectively. Moreover, a hot water dip followed by irradiation at 500 Gy totally eliminated the decay by *Alternaria alternate* for 8 days at 23°C. However, the combination of hot water with irradiation is less used due to detrimental effects on the quality of treated clementines, which causes contradictory results in different studies (Conway *et al.* 2004; Palou *et al.* 2007).

Biological control has some variations to reduce the decay compared to fungicides since biocontrol is easily affected by the environmental conditions. Through a combination of UV-C irradiation and biocontrol treatment, yeast *Candida oleophila* strain '13L' treatment, reduced the growth of *P. digitatum*. A combination of biocontrol treatment is not easy to handle due to variable environmental conditions and fastidious concentrations of the biocontrol antagonist. A study was conducted to evaluate the effect of gamma irradiation and *Pseudomonas flourescens* as biocontrol agents against *Penicillium expansum*. In general, *P. expansum* was completely inhibited at above 3 kGy. However, the suspension of *P. fluorescens* and *P. expansum* mixed in proportion of 3 : 5 (v/v) was observed after 48 h with 600 Gy of gamma irradiation. This shows that *P. expansum* was effectively inhibited at around 600 Gy, indicating that the combination of *P. fluorescens* with gamma irradiation shows an effective method for control of postharvest disease, *P. expansum* (Mostafavi *et al.* 2011).

Another combination is with chlorination. At present, chlorination is one of the few chemical agents used to disinfect postharvest diseases. This is effective and relatively inexpensive. Mainly, it is used during harvesting, farm handing, storage, marketing, transportation, and processing. Sodium dichloro-s-triazinetrione (NaDCC), a chlorine donor, and eco-friendly chemical, has been approved by the United states Environmental Protection Agency and the World Health Organization for the routine of drinking water (Clasen and Edmondson 2006). Treatment of NaDCC reduced the initial microbial load in various vegetables of up to 1.69 to 2.42 log compared to water treated control. Although NaDCC has antibiotic activity, it does not have the ability to maintain efficiency during storage. A combination of gamma irradiation with NaDCC treatment showed a synergistic effect on the disinfection of *B. cinerea* and *B. elliptica*. The sensitivity was dramatically increased with the combined treatment compared with gamma irradiation alone (Yoon *et al.* 2014; Kim and Yun 2014a). Based on artificial inoculation, the combined treatment with 0.4 kGy of gamma irradiation and 30 $\mu g\,l^{-1}$ of NaDCC completely inhibited the growth of *B. cinerea* on paprika (Fig. 3). The combined chlorine-ionizing radiation disinfection increases the synergistic effect, indicating that the minimum dose of the developmental inhibition on *B. cinerea* was reduced from 2 kGy to 0.4 kGy irradiation with 30 $\mu g\,l^{-1}$ of NaDCC. This contributed to the preservation of the physical properties in irradiated paprika (Fig. 3).

Instead of using NaDCC for combination with irradiation, nano-silver particles and nano-sized silica silver (NSS) were used as a combined treatment with gamma irradiation for disinfection of *B. cinerea* and *B. elliptica* in apples, pears, and lily (Jung *et al.* 2014; Kim and Yun 2014b). Several studies showed the inhibitory nature of nano Ag particles associated with sterilization (Park *et al.* 2006; Kim *et al.* 2011). Gamma irradiation showed no antifungal activity at a dose of 1.0 kGy, but combined treatments, NA or NSS at above 1.0 $\mu g\,l^{-1}$, at a dose of 1.0 kGy showed the strongest antifungal activity.

Quality evaluation of irradiated postharvest

Irradiation treatment has not been found to affect human health. There is a concern that radiation treatment affects not only plant pathogens, but also the physical properties and nutrition of postharvest. Mostly, irradiation is applied below I kGy due to the tolerance of fresh fruits and vegetables, because over a 1 kGy dose severely affects their physical properties. Most studies have suggested that nutrient losses of irradiated postharvest do not occur in fruits at the optimal dose for phytosanitary security (Akhter *et al.* 2005; Akhter *et al.* 2008). Irradiation at optimal levels (0.30 kGy) did not affect the ascorbic acid (Akhter *et al.* 2008). Irradiation dose at up to 250 Gy did not show any negative impact on the vitamin C content of bananas, and at up to 600 to 800 Gy did not affect the sugar content, vitamin C, or protein content of bananas. In general, the quality of the proteins, lipids, and carbohydrates is not affected by irradiation. The effects of nutrients by ionizing radiation depend on the radiation dose and modifying factors such as temperature, humidity, and the presence of oxygen. The irradiation of postharvest led to a reduction in firmness (Palekar *et al.* 2004; Jung *et al.* 2014;

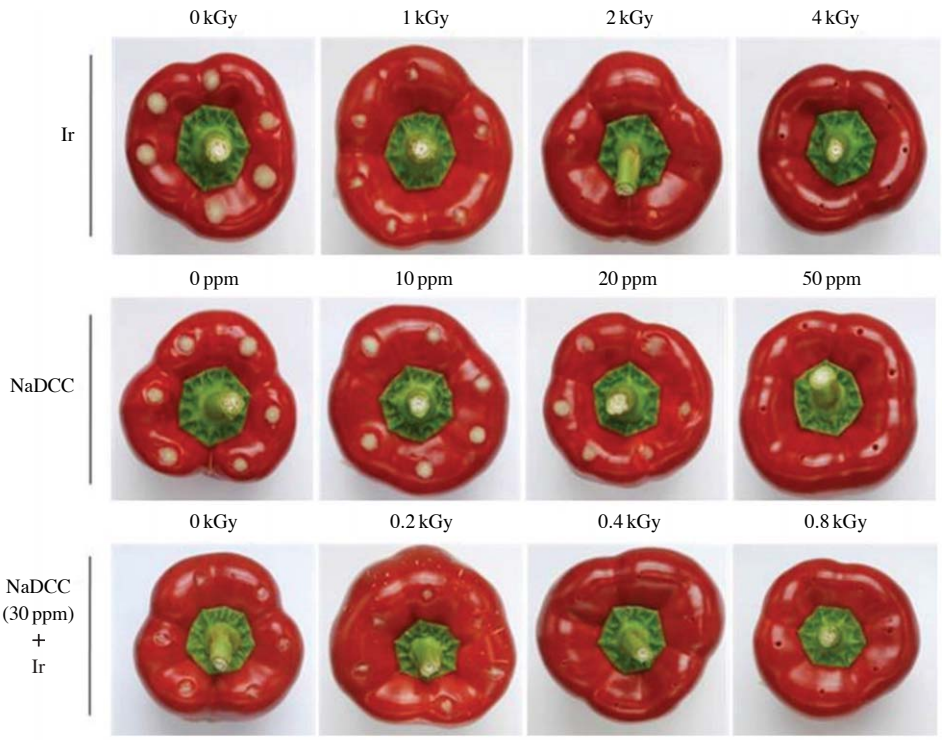

Fig. 3. Synergistic effect of combined treatment on *B. cinerea*. The fungal pathogenicity following treatment at over 2 kGy irradiation; conidia were not developed. In addition, the effect of chlorination on *B. cinerea* conidia showed that the treatment of 50 $\mu g\,l^{-1}$ of NaDCC leads to fungal disinfection. The combined treatment of 0.4 kGy irradiation and 30 $\mu g\,l^{-1}$ NaDCC fully inhibited the fungal development (Yun *et al*. 2014).

Yoon *et al*. 2014). The maximum doses applied to fruits or vegetables range between 1 and 2 kGy, and its values depend on the type of products and resistant cultivars. Combined treatment can prevent softening of fresh produce with the use of reduced doses of irradiation. The firmness of the 1 kGy-irradiated paprika is decreased, but combined treatment with NaDCC, down 1 kGy to 0.4 kGy, alleviated the physical properties (Yoon *et al*. 2014).

Challenges of phytosanitary irradiation

Currently, the use of irradiation in international trade regarding phytosanitary issues is gradually increasing in a number of countries, commodities, and volumes. For the effective phytosanitary treatment, irradiation treatment should deeply consider several challenges. Although a lot of studies have concluded that irradiated fresh produces are safe and there are no health concerns, some consumers still believe that irradiated products are harmful and that radioactivity exist after irradiation. Once the energy passes through the foods, it only inactivates the plant pathogens and does not leave any residual radioactivity. Therefore, education is needed to improve the knowledge of irradiated food in the public. Several studies have indicated that educational activities increase consumer acceptance of irradiated foods (Nayga *et al*. 2004).

In general, phytosanitary irradiation is more expensive than other alternatives. The main reason for the high cost of irradiation is the initial or start-up costs of a treatment facility. However, phytosanitary irradiation is not used at an optimal level at present, and thus the per-unit costs are will decrease. US$3-6 million is required for a facility, depending on the size of the plant and the equipment to be used. In addition, the location of facilities should be as close to the producers as possible, or near a packinghouse to save time and costs associated with transportation. Facility approval should be controlled by both the nuclear regulatory and phytosanitary issues (IPPC 2003). Another approach is to redesign mobile cobalt-60 commercial irradiation facilities in tractor trailers for incorporation to packinghouses with a safe regulatory framework, resulting in lucrative phytosanitary irradiation capability in the future.

CONCLUSION

Losses caused by postharvest diseases are a very significant issue because the value of fresh fruits and vegetables increases several times from the field to the consumer. Postharvest diseases cause losses in a wide variety of crops in developing countries due to a lack of large storage facilities. Effective postharvest treatments against postharvest diseases of fruits and vegetables require new control methods (Karabulut and Baykal 2004). Although there are several alternatives to control postharvest diseases, they are limited due to concerns about human health risks and environmental issues associated with fungicide residues. Ionizing irradiation is a promising phytosanitary treatment that passes through fruits or vegetables to inactivate harmful pathogens. Compared to foods, very little research has been done to determine the effects of irradiation on postharvest pathogens found in fruits and vegetables. There are some factors that might affect the efficacy of irradiation, including oxygen concentration, host, temperature, dose rate, and type of pathogens. Thus, it is necessary to understand if any of the other factors are directly associated with postharvest produce. In addition, irradiation can improve the safety of fresh produce, and increase their shelf life. A disadvantage of phytosanitary irradiation is that it is not accepted by the organic industry. The single irradiation dose required to kill postharvest diseases also has a negative effect on physical properties such as the skin color, texture, and firmness of the stored fruits and vegetables. Therefore, combined treatment with other agents is required to decrease the dose effects on the physical properties. There are some barriers to overcome this in commercial irradiated products. It is required to prepare strategies for consumers to overcome their fear of irradiated products. Another challenge of phytosanitary irradiation is cost because commercial use still does not meet the current economy of scale. Finally, it is worth emphasizing that phytosanitary irradiation is still unfamiliar compared to other treatments in postharvest diseases, but its progress and effectiveness made in this area during the last several decades has been remarkable. It was designed to provide plant quarantine security such as pre-clearance inspections. There is no doubt that if the challenges to irradiation treatment are well handled, phytosanitary irradiation will be the ideal technology for the control of postharvest diseases of fruits and vegetables, and will offer the possibility of accessing high export markets in the future.

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