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**PLANT PATHOLOGY**

# Irradiation as an Alternative Method for Post–harvest Disease Management: An Overview

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**ABSTRACT**

Considering the high economic worldwide loss due to the post-harvest decay of fruit and vegetables, and the frequent development of pathogen isolates that are resistant to synthetic fungicides, there is a need to develop more eco-friendly alternatives for controlling post-harvest diseases. Irradiation (Gamma and UV-C) is one such physical method which is promising, but when used separately is not as effective as fungicides. Therefore, to improve its effectiveness and persistence, irradiation method can be combined with other complementary control measures such as sodium carbonate, heat treatment, chemicals, cold storage and bio-control agents as apart of integrated disease management strategies. Low doses of UV radiation, particularly UV-C have proven to be effective in delaying ripening and senescence, diminishing decay and even in increasing the content of beneficial compounds owing to its germicidal properties. It mainly inactivates microorganisms, especially pathogens by creating damage in the genetic material (DNA) of the cell. These methods can be efficiently used to delay the ripening of fruits, to inhibit germination, improve nutritional quality, minimize insect infestation and deactivate viruses. Short shelf life of mushroom can be extended by inhibiting cap opening and browning, stalk elongation, reducing the level of microbial contamination and finally by increasing the concentration of vitamin $D_2$ significantly, without causing any adverse effect on its taste. This review deals with the information of maximum benefit which can be achieved from irradiation technology, and when combined with other chemical and physical methods with integrated strategy will provide synergistic effects for post-harvest disease control.

**Highlights**

- Reduction of post-harvest loss is a critical component in ensuring global food security
- Irradiation technology stands effective and as a safer alternate to synthetic fungicides

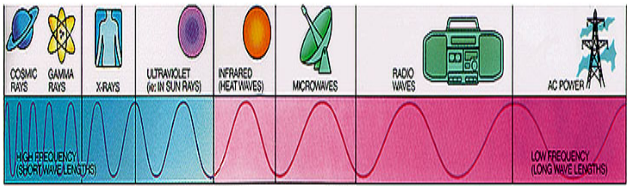
**Keywords**: Irradiation, post-harvest disease, ionizing radiation, UV-C, Gamma radiation

Post-harvest loss is a global shame considering 870 million people who are undernourished and the millions of others suffering from nutrient deficiencies. Estimates of post-harvest loss range from 10 to 50% depending upon the commodity and the country (ElGhaouth *et al.* 2004). This happens because of microorganisms (fungal and bacterial), rodents, insects and breakdown of the produce as a result of environmental conditions. In developing countries, 50% or more of the fresh produce can be lost because of fungal spoilage (Feliziani and Romanazzi 2013). In the storage of fruit and vegetables post-harvest rot is the major problem. This can be reduced by improving the post-harvest management and its' processing. Earlier chemicals have been used to control these pests, but because of their harmful effect on human and nature they have been removed. Besides, the appearance of pathogen strains that are resistant to synthetic fungicides has been reported for many fungi (FRAC

2012). In addition, over the last few years, there has been increasing number of requests from consumer for fresh fruit and vegetables that are free from fungicide residues. Consequently, alternative control strategies, i.e., antagonists, natural compounds and physical treatments have attracted them. The reduction of post-harvest food loss is a critical component of ensuring future global food security which is again a critical component of integrated resource management (Elangbam *et al.* 2015). Meanwhile Irradiation (Gamma and UV-C), physical treatment, which has shown reduction in post-harvest decay for several fruits and vegetables (Cia *et al.* 2007) is more effective when integrated with other biologically based technologies. The effect of irradiation on post-harvest disease management depends upon the dosage of irradiation used and the pathogen against which we are using it. Therefore, this article reviews the importance of irradiation for controlling the post-harvest diseases of fruit and vegetables describes the effect of irradiation as well as its' combination with other treatments such as sodium carbonate, heat treatment, chemicals, cold storage and bio-control agent.

## Irradiation in nutshell

Irradiation is a process of controlled application of energy from ionizing and non-ionizing radiations to increase the safety and shelf-life of food. In the United States, the Food and Drug Administration, has approved the use of irradiation for beef, pork, poultry, molluscan shellfish, shelled eggs, fresh fruit and vegetables, lettuce, spinach, spices, and seeds. The irradiation process has also been approved by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Codex Alimentarius Commission. About 100 countries have approved this process in more than 100 food items. India first approved them in 1994 (Hemant Lagvankar). Irradiated fresh commodities carry the "radura" symbol placed on the "country of origin" or product locator unit (PLU) stickers, and a statement about its treatment with irradiation. Ionizing and non-ionizing radiations are physical treatments that can be used for the control of post-harvest diseases. Irradiation has been used to delay ripening-associated processes and to control pathogens and insects. Different types of radiation have been tested for fruits. The primary mode of action of many physical treatments acts as a disinfection of the commodity. Thus, fungal spores and mycelial infections both on and in the outer cell layer of fruits or vegetables are either removed or destroyed. A complex defense response against the pathogens arise in the irradiated tissue, which includes the induction of a wide range of genes, the modification of enzyme activities, as well as *de novo* synthesis of pathogenesis related proteins (PR) and the increase of secondary metabolites (Charles *et al.* 200; Liu *et al.* 2011; Colas *et al.* 2012 and Civello *et al.* 2014).




*Ionizing radiations* *Non-Ionizing Radiations*

**Fig. 1:** Electromagnetic Spectrum

## Ionizing Radiation

Ionizing radiation includes radioactive ($^{60}Co$ or $^{137}Cs$, $\gamma$-rays) and machine sources [electron beams ($\beta$ particles) and X-rays]. Irradiation of fresh foods, including fruit and vegetables at a dosage not to exceed 1000Gy (100 krad) was approved by the United States Food and Drug Administration, US FDA (21 CFR Part 179.26) and was amended to include an energy level of ≤ 7.5 MeV for X-rays using Tantalum or gold as a target material (US FDA 1986, 2004). Uses of ionizing radiation of fruits and vegetables include insect disinfestations, sprout inhibition, control of human pathogens, and maintenance of quality during storage, improvement of nutritional or functional components and the control of post-harvest diseases (Kader 1999 and Patil 2004).

## Non-ionizing Radiation

Non-ionizing radiation has actual potential amongst the physical methods for controlling post-harvest diseases. Ultraviolet radiation is a non-ionizing radiation which ranges between X-ray and visible light of the electromagnetic wave spectrum. It is one of the presently available non-fungicidal strategies to control post-harvest diseases (Canale *et al.* 2011; Darras *et al.* 2012). The UV light present in the sunlight (Fig. 2) is generally designated as UV-C (short wavelength, below 280 nm), UV-B (middle wavelength, 280-320 nm), and UV-A (large wavelength, 320-390 nm). Although low in energy and the lack in penetrating power affects a wide range of biological processes, it has been reported to stimulate numerous plant responses (Mau *et al.* 1998; Ait Barka *et al.* 2000; Ben-Yehosua, 2003 and Kasim *et al.* 2008). Post-harvest treatment of fruit and vegetables with relatively low dosage of UV radiation, particularly UV-C, has proven to be effective to delay ripening and senescence, diminishing decay and even in increasing the content of beneficial compounds (Shama *et al.* 2005; Charles *et al.* 2007) owing to its germicidal properties.

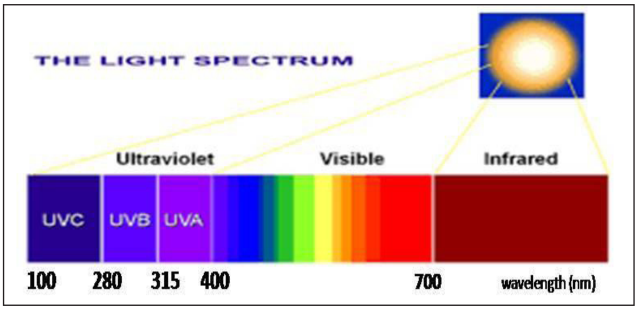



**Fig. 2:** (Photo:http://www2.gov.bc.ca/gov/content/health/keeping-bc-healthy safe/radiation/ultraviolet-uv-radiation)

## Alternatives to synthetic fungicides

Efforts of researchers have led to the development of novel control tools that can be used as alternatives to synthetic fungicides. This kind of pre-harvest application can be grouped into three categories: (i) natural compounds; (ii) decontaminating agents that are 'generally recognized as safe' (GRAS); and (iii) biological control agents (BCAs). All of these tools can be used alone or as combinations, to benefit from additive or synergistic effects (Mari *et al.* 2009; Romanazzi *et al.* 2012).

## How Does Irradiation Work

Irradiation kills microbes primarily by destroying DNA. Hence, prevents proliferation of microorganisms such as bacteria, viruses, mold, and yeast (Fig. 3). The sensitivity of the organism increases with the complexity of the organism. Thus, viruses are most resistant to destruction by irradiation, while insects and parasites are more sensitive. Spores and cysts are quite resistant to the effect of irradiation because they contain little DNA and are in a highly stable resting state (Temur and Tiryaki 2013). The effect of irradiation depends on the fungus type, application dose, oxygen, moisture content, composition of food, and storage conditions (Monk *et al.* 1995; Frazier and Westhoff 1988; Smith and Pillai 2004; Kabak and Var 2005; Aziz *et al.* 2006). Bacterial spores are more resistant to ionizing radiation than those of the vegetative cells (Table 1). Gram-positive bacteria are more resistant than gram-negative bacteria. The resistance of yeasts and molds vary considerably, but some are more resistant than bacteria.

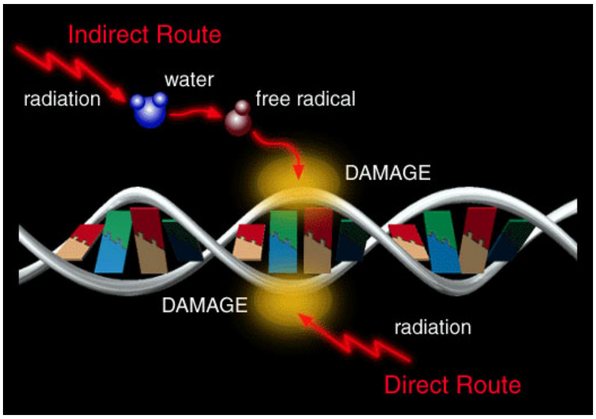



**Fig. 3:** Destruction of DNA strands by Radiation

Gray (Gy) **-** The unit (or level) of energy absorbed by food from ionizing radiation as it passes through its processing.

1 Gray (Gy) = 100 rads

1,000 Gy = 1 Kilogray (kGy)

Rad - Another name or unit for "radiation energy absorbed" by food being processed with radiation.

1,000 rads = 1 Kilorad = 10 Grays

1,000,000 rads = 1 Mrad = 10 kGy

(Rad has been superseded by Gray)

**Table 1:** Approximate Killing Doses of Ionizing Radiations in Kilorays (kGy)*

| Organism | Approximate lethal dose (kGy) |
|---|---|
| Insects | 0.22 to 0.93 |
| Viruses | 10 to 40 |
| Yeasts (fermentative) | 4 to 9 |

**Adapted from: Institute of Food Technologists' Expert Panel on Food Safety and Nutrition. 1983*

| | |
|---|---|
| Yeasts (film) | 3.7 to 18 |
| Molds (with spores) | 1.3 to 11 |
| **Bacteria (cells of pathogens)** | |
| *Mycobacterium tuberculosis* | 1.4 |
| *Staphylococcus aureus* | 1.4 to 7.0 |
| *Corny bacterium diphtheriae* | 4.2 |
| *Salmonella* spp. | 3.7 to 4.8 |
| **Bacteria (cells of saprophytes)** | |
| ***Gram-negative*** | |
| *Escherichia coli* | 1.0 to 2.3 |
| *Pseudomonas aeruginosa* | 1.6 to 2.3 |
| *Pseudomonas fluorescens* | 1.2 to 2.3 |
| *Enterobacter aerogenes* | 1.4 to 1.8 |
| ***Gram-positive*** | |
| *Lactobacillus* spp. | 0.23 to 0.38 |
| *Streptococcus faecalis* | 1.7 to 8.8 |
| *Leuconostoc dextranicum* | 0.9 |
| *Sarcinalutea* sp. | 3.7 |
| ***Spore producer Bacteria*** | |
| *Bacillus subtillus* | 12 to 18 |
| *Bacillus coagulans* | 10 |
| *Clostridium botulinum* (A) | 19 to 37 |
| *Clostridium botulinum* (E) | 15 to 18 |
| *Clostridium perfringens* | 3.1 |
| *Putrefactive anaerobe* 3679 | 23 to 50 |
| *Bacillus stearothermophilus* | 10 to 17 |

* *Adapted from Frazier, W.C. and Westhoff, D.C. 1988*

## Gamma radiation for controlling post-harvest diseases

The penetrating power of gamma-rays is more than fungicides thereby serving them to reach microorganisms inside the fruits of different sizes and shapes (Jarrett 1982) which are not accessible to chemicals. The effectiveness of irradiation to control post-harvest diseases depends on the pathogen, its growth stage and the number of viable fungal cells on or within the tissue (Kader *et al.* 1986). It is well established that irradiation disinfestations require lower ionizing radiation doses than the decontamination offood, which might call for higher doses. Gamma irradiation at 0.75 and 1 kGy inhibited conidial germination and mycelia growth *in vitro* of *Colletotrichum gloeosporioides*. Similarly doses of 0.75 and 1 kGy reduced anthracnose incidence and severity in 'Golden' papaya fruits (Cia *et al.* 2007). The effects of irradiation dose and refrigerated storage conditions on 'Nagpur' mandarin, 'Mosambi' sweet orange and 'Kagzi' acid lime was evaluated and a dosage up to 1.5 kGy was found to be effective in delaying *Penicillium* rot, while turning to be ineffective in controlling *Botryodiplodia theobromae* and *Alternaria citri* (Ladaniya *et al.* 2003). Anthracnose severity on 'Keitt' mango fruits was reduced by doses equal to or above 0.5 kGy and rot incidence reduced by 0.75kGy dose (Spalding *et al.* 1988). Dosage of $\gamma$-irradiation (0.06- 0.5 kGy) inhibited cap opening and browning, stalk elongation, reduce in the level of microbial contamination (surface molds), and extend the shelf-life of mushrooms (Lescano 1994). Irradiation at doses below 1 kGy is an effective insect disinfestation treatment against various insect species of quarantine significance in marketing fresh fruits and vegetables (Kader 1986).

## UV-C radiation for controlling post-harvest diseases

Amongst physical methods, Non-ionizing radiation has a potential for controlling post-harvest diseases (Wilson *et al.* 1997). Low dosage of short-wave ultraviolet light (UV-C, 190–280 nm wavelengths)can control storage rots of many fruits and vegetables (Terry *et al.* 2004) by targeting the DNA of micro-organism hence is used as a germicidal or mutagenic agent. In addition to this direct germicidal activity, UV-C irradiation can modulate induced defense in plants (Terry *et al.* 2004; Shama *et al.* 2005). At appropriate wavelength and dose rates, UV-C irradiation can stimulate accumulation of stress-induced phenylpropanoids (Ben-Yehoshua *et al.* 1998) and pathogenesis-related proteins (Porat *et al.* 2000). The effect of UV radiation in reducing green mould in grapefruit is mediated through the host response, rather than being the only result of the germicidal action of the UV treatment (Droby *et al.* 1993).

Later, an increase in grapefruit resistance to *P. digitatum* was attributed to chitinase and $\beta$-1, 3-endoglucanase induction in the fruit skin (Porat *et al.* 1999). The treatment can also extend the post-harvest life of fruits by delaying ripening. The occurrence of *Alternaria alternata*, *Botrytis cinerea* and *Rhizopus stolonifer* in tomato were reduced with different UV-C dosages, and these fruits were firmer in texture and less red in colour than those used as controls (Liu *et al.* 1993). UV-C treatment significantly reduced Botrytis storage rot (*Botrytis cinerea*) in cv. 'Pajaro' of strawberries at UV has a dosage of about 0.50 and 1.00 kJ/$m^2$ while the. Single table grape berries (cv. Italia) at 0.125 to 0.5 kJ/$m^2$ in comparison with the un-irradiated control (Nigro *et al.* 1998 and 2000). The effect of different types of UV radiations on *in vitro* and *in vivo* growth of *Colletotrichum musae*, causing anthracnose disease of banana were investigated and UV-C irradiation was found to be the most effective in inhibiting the fungal growth (Bokhari *et al.* 2013). The same fact was observed for citrus black spot, caused by *Guignardia citricarpa* (Canale *et al.* 2001). UV-C light (254 nm) at 79.2 $kJm^{-2}$ and 105.6 $kJm^{-2}$ reduced the mycelial growth *Fusarium solani* f.sp. *eumartii*, causal agent of potato dry rot. A research was carried out on the effect of the orientation of fruits of post-harvest commodities following low dose of ultraviolet light-C treatment on host induced resistance to decay (Stevens *et al.* 2005). The possibility of inducing resistance to bitter rot (*Colletotrichum gloeosporioides*), brown rot (*Monilinia fructicola*), and green mould (*Penicillium digitatum*) in 'Golden delicious' apples, 'Elberta' peaches, and 'Dancy' tangerines, respectively, by treating them with ultraviolet light-C at the stem end in a stationary position without rotation were compared with the conventional procedure where fruits were rotated four times, thereby exposing the entire surface area to the full effects of the UV-C light.

Results revealed that when the ends of the fruits stem were treated with UV-C light in a stationary position, they resisted post-harvest decay. This is equal to or slightly better than the result of the fruits that were rotated four times. However, when the fruits were rotated, exposing only one or two different sides to UV-C light, susceptibility to decay appeared to increase. UV-C treatment at both 1.5 and 3 $kJm^{-2}$ compared to non-treated fruits could increase the post-harvest life of `Karaj´ persimmon by reducing the post-harvest disease incidence during storage, due to its direct effect on fruit physiology or fungal spores. In order to have suitable firmness, it must be integrated with other effective post-harvest treatments (Khademi *et al.* 2013). UV-C irradiation could be used as an effective and rapid method to preserve the post-harvest life of Ripe 'Tommy Atkins' mangoes without adversely affecting certain attributes (Gonzalez *et al.* 2001). They showed that UV-C irradiation for 10 min was the most effective treatment in suppressing decay symptoms and maintaining firmness during storage at 5 or 20 °C. Such fruits maintained better visual appearance and showed greater levels of putrescine and spermidine after cold storage than non-irradiated controls. UV-C treatment of 'Aroma' strawberries delayed fruit softening, which could be related to the decrease of the transcription of a set of genes involved in cell wall degradation, during the first hours after the treatment (Pombo *et al.* 2009). Post-harvest treatments of cv. 'Napoleon' of table grapes with UV-C light increases health promoting phenolics namely resveratrol (Cantos *et al.* 2000).

### Irradiation of Mushrooms through UV-C

UV-C radiation could potentially be used for sanitizing fresh mushrooms and may act as a useful non-chemical way of maintaining mushroom's quality and extending their post-harvest shelf-life (Beaulieu *et al.* 2000). The effects of UV-C light (0.45–3.15 $kJm^{-2}$) was investigated in the inactivation of *Escherichia coli* O157:H7 and the inhibition of lesion development on the mushroom surface (Guan *et al.* 2012). UV-C treatment (4 $kJm^{-2}$) enhanced antioxidant enzyme activities (catalase, superoxide dismutase, ascorbate peroxidase and glutathione reductase) in shiitake mushrooms (*Lentinus edodes*) (Jiang *et al.* 2010). The result of some recent works on the UV application of the post-harvested fruits and vegetables, its direct germicidal activity and its hormectic effects were reviewed (Ribeiro *et al.* 2012). Pulsed UV light (PUV) can be very effective in rapidly increasing the vitamin D2 content of fresh *Agaricus bisporus* (Kalaras *et al*. 2012) and adenosine, cordycepin, polysaccharides and antioxidant activity in *Cordyceps militaris* (Purwidyantri *et al.* 2012, Kalyani and Manjula 2013). A comprehensive study was done (Fernandes *et al.* 2012) on irradiations effect on physio-chemical and bio-chemical parameters of different species of mushrooms.

### Combination of irradiation with other treatments against the post-harvest diseases

Recently, combined treatment is recommended to control the post-harvest diseases. The main purpose of combination is to increase the effectiveness (Conway *et al.* 2004) and to decrease the negative effect of application by exposing it to lower doses when compared to single application. The effect of irradiation is more promising when applied in combination with hot water treatment, chemicals such as $SO_2$ fumigation and cold storage treatment (Beraha *et al.* 1960; Tiryaki *et al.* 1994). An extensive research work is also done based on the evaluation of physical means used alone or in combination with other control methods for citrus decay control is reviewed (Palou 2009). Sodium carbonate (SC) treatment is yet another alternative combination against the post-harvest diseases because it is inexpensive, readily available, and can be used with a minimal risk of injury to the fruits. The effects of the integration of sodium carbonate (dips at 20°C for 150s in aqueous 3% sodium carbonate solutions) treatments and X-ray irradiation (at doses of 510 and 875 Gy) for *Penicillium* decay control on 'Clemenules' clementine mandarins was observed and the result showed that treatments (irradiation and SC), especially at X-ray highest dose 875 Gy, significantly reduced disease incidence and the severity of both green and blue moulds (*Penicillium digitatum* and *Penicillium italicum)* at 5°C (Palou *et al.* 2007).

However, these reductions were not high enough for satisfactory disease control under hypothetical commercial conditions and it is concluded that the combination of treatments could not be a substitute for the synthetic fungicides.

In contrast to fungal growth, pathogen sporulation especially that of *P. digitatum* was clearly inhibited on inoculated Clementine by the combined treatments. Since sodium carbonate does not exert anti-sporulant activity, this effect should be attributed to irradiation.

### Combination with heat treatment

The integration of curing treatments (35°C for 72h) or hot water dips (50-55°C for 2 min) with UV-C illumination was found superior to either the treatment alone in reducing decay or in maintaining the fruits quality (Ben Yehoshua *et al.* 2005). Previous application of heat significantly reduced the risk of rind damage due to UV-C exposure. When UV- C treatment preceded heat treatment, the elicitation of phytoalexins in fruit rind was inhibited. Dipping fruit in water at 52°C for 5 mins followed by gamma irradiation at low dose (500Gy) delayed the appearance of green mould of citrus (*Penicillium digitatum)* by upto 40 days (Barkai-Golan *et al.* 1969).

### Combination with cold storage

Combination of irradiation with cold storage is more promising **(**Beraha *et al.* 1960; Tiryaki and Maden 1991). In a study carried out by Tiryaki (1990), the degree of sensitivity of these storage pathogens on PDA to gamma-rays at 3 to 4°C was found (from resistant to sensitive): *B. cinerea* > *Alternaria tenuissima* > *P. expansum* > *R. stolonifer.*

### Combination with biocontrol agents

Bio control agents are yeast, bacteria or other filamentous fungi which are capable to colonise rind infection sites and offer effective prevention activity against pathogens that may reach the treated fruit during storage and commercialization (El-Ghaouth *et al.* 2002; Janisiewiez and Korsten, 2002). D'hallewin *et al*. (2005) studied on the combination of ultraviolet-C irradiation and bio control treatments to control decay caused by *Penicillium digitatum* in orange fruit. The combination of yeast *Candida oleophila* with UV-C irradiation evidenced a synergistic effect in reducing *Penicillium digitatum* mould whereas, no synergistic effects were observed when UV-C was combined with bacteria *Bacillus subtilis.* UV-C and Gamma rays reduced storage rot and delayed the ripening of peach fruits, but the combination of UV and gamma radiation showed no advantage over the use of either the UV or the gamma treatments alone (Lu *et al.* 1993).

## CONCLUSION

Post-harvest loss in fruit and vegetables is very high due to the lack of inadequate storage, preservation facilities and the adverse climatic conditions. Therefore, reduction of post-harvest loss is a critical component in ensuring future global food security. Irradiation technology promises to offer an effective mean for minimizing such loss and it also provides an effective and safer alternate to synthetic fungicides that are being phased out because of ecological or human health concerns. Radiation technology can complement and supplement existing technologies and can be efficiently used for insect disinfestations, sprout inhibition, maintenance of nutritional or functional components during storage and finally for controlling the post-harvest diseases. Although this technology has received increased attention, parameters like irradiation dose, intensity, type of produce and pathogen should be considered otherwise it may cause acute radiation injury (as dose increases) or other detrimental effects. Several issues regarding the wider use of irradiation for post-harvest treatment include the high costs coupled with poor consumer acceptability due to the lack of knowledge in relation to safety and benefits of irradiated foods. A majority of consumers will buy high-quality irradiated food and the percentage increases with increasing awareness hence a wider adoption of the technology. In order to fetch maximum benefit, irradiation technology has to be combined with other chemical and physical methods which will provide synergistic effects for the control of post-harvest diseases of fruit and vegetables.

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