African Journal of Agricultural Research Vol. 8(5), pp. 421-434, 15 February, 2013
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11x.004
ISSN 1991-637X ©2013 Academic Journals

Review

Irradiation alone or combined with other alternative treatments to control postharvest diseases

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Accepted 17 December, 2012

The postharvest diseases are considered worldwide as the most significant issue for postharvest facilities. Although there are various methods to decrease postharvest losses, consumers are looking for agricultural product free of chemicals. It is therefore necessary to develop alternatives to synthetic chemical control to reduce environmental risks and raise consumer confidence. Several alternatives such as food irradiation show promise, but none alone is as effective as fungicides. A strategy must be developed that combines several of these alternatives to enhance their effectiveness. Therefore, there is a need for a method combining couple of methods together. A combination for this purpose can be irradiation with other treatment such as, heating, cooling and sodium carbonate/sodium bicarbonate treatment. The safety of irradiated food is declared by joint FAO/JAEA/WHO Expert Committee for food irradiation. In this study, advantages and disadvantages of irradiation, and combination with various other treatments were evaluated and recommendations were provided to minimize the postharvest losses.

Key words: Irradiation, postharvest disease, combined treatment.

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) estimated that 25% of all food products are wasted after harvest worldwide. The most economic losses of foods are due to infestation with insects, fungal contamination and premature germination (Harris, 1998; Braghini et al., 2009a). Postharvest diseases also limit the storage period and marketing life of fruit. Postharvest losses are 5 to 10% when postharvest fungicides are used; without fungicides, losses of 50% or higher have occurred in some years (Margosan et al., 1997). Postharvest losses are estimated to be 30 to 40% in Turkey and sometimes it may reach to 50% (Anonymous, 2011).

There are some registered fungicides such as fludioxonil and azoxystrobin in the USA for postharvest application to control decay in products. However, postharvest use of these fungicides in most European Union countries and Turkey are prohibited due to fungicide regulatory issues. In addition, public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues, as well as the development of resistance of some pathogens to fungicides limit the postharvest application of chemicals to agricultural products (Karabulut and Baykal, 2004). Many of the fungicides such as benzimidazole and dicarboximide, that are still available for use, are losing their effectiveness because of the development of resistance in postharvest pathogen of *Botrytis cinerea* (Lennox and Spotts, 2003).

It is necessary to find alternatives to control postharvest pathogens to reduce environmental risks and raise consumer confidence. Various methods have been investigated, and although they show promise, none alone has been found to be as effective as fungicides. Therefore it is necessary to develop a strategy which combines several of these alternatives that may equal the effectiveness of fungicides (Conway et al., 2005). One of the promised alternative methods is the use of gamma irradiation with the combination of other treatments such as antagonists, natural compounds, and physical treatments (Cia et al., 2007).

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Table 1. Permitted radiation dose for food irradiation in Turkish Food Codex (Anonymous, 1999).

Food type	Aim of irradiation	Maximum dose (kGy)
Bulb, root and tuber	İnhbition of shooting, germination, and budding during storage	0.2
Fresh vegetable and fruit	Delay maturation	1.0
	To prevent insects	1.0
	Extend the shelf life	2.5
	The quarantina control	1.0
Grain, milled grain products, nuts, oil seeds, legumes, dried fruit and vegetables	To prevent insects	1.0
	Reducing microorganisms	5.0
	Extend the shelf life	5.0
Diried vegetable, spices, dried herbs, condiments and herbal tea	Reducing some pathogenic microorganisms	10.0
	To prevent insects	1.0

The Joint FAO/ IAEA/ WHO Expert Committee for Food Irradiatin (JECFI) concluded that foods irradiated up to 10 kGy (1 Gy=100 rad) are safe and nontoxic (WHO, 1981). This limit is adapted to Codex Standard in 1983 (Anonymous, 1987). Later on, JECFI, for the evaluation of toxicological, nutritional, chemical and physical aspects of foods, declared that irradiated up to 10 kGy are safe and nutritionally adequate as long as they are produced according to good manufacturing practices (WHO, 1999). The limit of 10 kGy is also accepted for Turkish Food Codex in 1999 (Anonymous, 1999). Irradiation of fresh foods including fruit and vegetables is permitted to be irriadated at doses up to 1000 Gy (US FDA, 2004). “Food Irradiation Regulation” was published in Turkey in 1999. Agricultural products permitted for irradiation treatment were listed in this regulation (Table 1).

All foods are radioactive to some extent as a result of exposure to natural background radiation. Irradiation of food does not induce additional radioactivity, because the sources of radiation approved for use in food irradiation are limited to those producing energy too low to induce sub-atomic particles (Anonymous, 2000). Chain reactions cannot occur; therefore, no radioactivity is added. Neither the food nor the packaging materials become radioactive (Urbain, 1986). It is physically impossible for irradiated food to be radioactive just as your teeth are not radioactive after you have had a dental X-ray. Irradiation is radiant energy. It disappears when the energy source is removed (Brennand, 1995). It is concluded that gamma-rays with the energy of 5 MeV and accelerated electrons with the energy of 10 MeV, even if high doses, does not causes any radioactivity. Cobalt-60 (^{60}Co) and Cesium-137 (^{137}Cs) are generally used for irradiation purposes, with the energy of 1.33 and 0.66 MeV, respectively. Thus, radioactivity is not possible even if the high doses are used with these irradiation sources (Anonymous, 1999; CAC, 2003; Farkas, 2006). Radioactivity of food is only possible with the exposure to radioactive particle leak caused by nuclear accident and nuclear weapon tests (Çelebi, 2007). About 170 gamma facilities exist worldwide. Most facilities are used for medical sterilization, surgical or the preparation of packaging materials (Shea et al., 2000).

The ultimate goal of this review article is to devise a strategy that combines several of alternatives below mentioned that will equal the effectiveness of chemical control. The specific objective of this paper is to determine the effect of irradiation alone and in combination with other treatments such as sodium carbonate/sodium bicarbonate, heat treatment, chemicals, modified atmosphere packaging, cold storage and biocontrol agent.

IRRADIATION TREATMENT AGAINST THE POSTHARVEST DISEASES

Microbes in food fall into three categories. Some micro-rganisms, such as those that produce fermentation, create desirable changes in foods. Spoilage micro-organisms change the color, odor, and texture of food, rendering it unpalatable, but they do not cause human illness. Pathogens cause human disease and include invasive and toxigenic bacteria, toxigenic molds, viruses, and parasites. All food production techniques from the farm to the table are concerned with minimizing spoilage, eliminating pathogens, and prolonging shelf life. Gamma irradiation can contribute for the reduction of postharvest losses caused by fungi and reduce the use or doses of fungicides on disease control (Cia et al., 2007).

Irradiation has been used for the preservation and production of foods that are free of pathogenic micro-organisms and is therefore an important tool for the control of food contaminating microorganisms. In another words, irradiation of foods can reduce the risk of foodborne illness (Rustom, 1997; Braghini et al., 2009a).

This approach has also contributed to reduce economic losses resulting from food deterioration and to increase food safety, thus favoring the acceptance of products exported by developing countries (Loaharanu, 1994). Food irradiation is a process by which food is exposed to a controlled source of ionizing radiation to prolong shelf life and reduce food losses, improve microbiologic safety, and/or reduce the use of chemical fumigants and additives. It can be used to reduce insect infestation of grain, dried spices, and dried or fresh fruits and vegetables; inhibit sprouting in tubers and bulbs; retard postharvest ripening of fruits; inactivate parasites in meats and fish; eliminate spoilage microbes from fresh fruits and vegetables; extend shelf life in poultry, meats, fish, and shellfish; decontaminate poultry and beef; and sterilize foods and feeds (Brennand, 1995).

The dose of the ionizing radiation determines the effects of the process on foods. Radiation doses are measured in international units called Gray (Gy). Food is irradiated at levels from 50 Gy to 10 kGy, depending on the goals of the process. Low-dose irradiation (≤1 kGy) is used primarily to delay ripening of produce or kill or render sterile insects and other higher organisms that may infest fresh food. Medium-dose irradiation (1 to 10 kGy) pasteurizes food and prolongs shelf life. High-dose irradiation (>10 kGy) sterilizes food. The FDA has authorized the following 4 sources of ionizing radiation for food treatment: ^{60}Co, ^{137}Cs, machine-generated accelerated electrons not to exceed 10 MeV, and machine-generated X-rays not to exceed 5 MeV. All petitioners for FDA approval of food irradiation must satisfy technical requirements that limit dose and specify conditions under which the food will be irradiated.the technical effect on the food, dosimetry, and environmental controls must be defined and in compliance with the Federal Food, Drug and Cosmetic Act (Shea et al., 2000).

Ionizing radiation has been widely recognized as a method of decontamination of foodstuffs. Many reviews have summarized the nutritional adequacy of irradiated foods. They clearly demonstrate that irradiation results in minimal, if at all noticeable, changes in the taste, provided that the optimal dose for each type of food is not exceeded. In general, irradiation to the recommended doses changes the chemical composition of foods very little. At doses below 1 kGy, nutritional losses are considered to be insignificant, and none of the chemical changes found in irradiated foods is harmful, dangerous or even lying outside of the limits normally observed (Braghini et al., 2009b). Doses of up to 10 kGy are highly effective in microbial decontamination and have no adverse effects on the nutritional quality of cereal grains (WHO, 1994; Aziz et al., 2006).

The use of gamma radiation to inactivate aflatoxins was investigated. The toxicity of a peanut meal contaminated with Aflatoxin B_1 (AFB_1) was reduced by 75 and 100% after irradiation with gamma-rays at a dose of 1 and 10 kGy, respectively. However, doses higher than 10 kGy inhibited the seed germination, and increased the peroxide value of the oil in gamma-irradiated peanuts. The presence of water has an important role in the destruction of aflatoxin by gamma energy, since radiolysis of water leads to the formation of highly reactive free radicals. These radicals can readily attack AFB_1, at the terminal furan ring, giving products of lower biological activity. The mutagenic activity of AFB_1 in an aqueous solution (5 pg ml^{-1} water) was reduced by 34, 44, 74 and 100% after exposure to gamma-rays at 2.5, 5, 10 and 20 kGy, respectively. Also, a dose of 10 kGy completely (100%) inactivated AFB_1, and destroyed 95% of AFG_1 in a dimethylsulphoxide-water (1:9, v/v) solution (Rustom, 1997).

Sclerotia of *Whetzelinia sclerotiorum* obtained from field grown peas and from laboratory cultures were exposed to gamma radiation from a ^{60}Co source. Over 2500 sclerotia irradiated at levels from 100 to 800 krads were observed. Sclerotia with moisture levels below 10% were highly resistant to radiation damage having an LD_{50} of up to 600 krads. An increase in moisture content resulted in a marked decrease in the LD_{50} (Blanchette and Tourneau, 1977).

Some studies in the use of ionizing radiation to control *Botrytis* rot in table grapes and strawberries were performed by Nelson et al. (1959). Cultures irradiated with 4×10^5 rep (Röntgen Equivalent Physique) made no growth after transfer to unirradiated media. The rate of spread of *Botrytis* rot among grape berries and strawberries was markedly reduced at doses of 1×10^5 and 2×10^5 rep

In a study showed that an irradiation dose of 200 000 rep inhibited brown rot for 10 days at 80 to 85°F. Unirradiated peaches were completely rotted within 5 days (Beraha et al., 1959).

Irradiation doses for the inhibition of fungal development are presented in Table 2 for several fungi. The important criteria for the evaluation of irradiation treatment against the fungi are D_{10} values. This term is radiaton dose that causes 90% decrease in population. The lower the D_{10} values, the higher the sensitivity of microorganism. D_{10} values for *Aspergillus flavus* and *A. parasiticus* which are aflatoxigenic were specified as 0.25 and 0.31 kGy, respectively (Table 3) (TAEK, 2001).

Advantages of irradiation treatment

The problems caused by diseases have been maximized by the development of pathogen resistance to fungicides and by the withdrawal of some products from the market. Moreover, consumers are looking for fruit free of chemical residues. Consequently, alternative control strategies, such as antagonists, natural compounds, and physical treatments have drawn attention. Gamma and UV-C (254 nm) irradiations are physical treatments that

Table 2. Radiation dose for fungal inhibition at room temperature (TAEK, 2001).

Fungus	Irradiation medium	Radiation source	Dose (kGy)
Aspergillus flavus	% 0.1 pepton	Electrons	1.6
Aspergillus niger	Malt extrakt agar	Gamma-ray	2.5
Aspergillus parasiticus	Water	Gamma-ray	1.6
Alternaria spp.	Malt ekstrakt agar	Gamma-ray	6.0
Botrytis cinerea	Malt ekstrakt agar	Gamma-ray	5.0

Table 3. Comparison of D_{10} values for fungi irradiated in aquous suspension (TAEK, 2001).

Fungus	Irradiated with gamma-rays (kGy)
Aspergillus niger	0.245
A.flavus	0.250
Avicularia versicolor	0.282
A. parasitucus	0.310
Penicillium cyclopium	0.397
Alternaria alternata	2.409

can be used for the control of postharvest diseases Besides exhibiting fungicidal effects, these treatments can also induce resistance in fruit (Conway et al., 2005). Lu et al. (1993) reported that both UV-C and gamma-rays reduced storage rot and delayed ripening of peaches.

Gamma radiation is effective on all stages of the life cycle of a pest such as a fruit fly and it is ready to be used as an efficacious quarantine treatment (Cia et al., 2007). Green mold, caused by *Penicillium digitatum* and blue mold, caused by *P. italicum* are the most economically important postharvest diseases of citrus in Spain, California, and all citrus production areas characterized by low summer rainfall. Both diseases are primarily controlled worldwide by the application of synthetic fungicides such as imazalil, sodium ortho-phenyl phenate, or thiabendazole. However, alternative methods are needed because the widespread use of these agrochemicals in commercial packinghouses has led to proliferation of resistant strains of the pathogens (Kinay et al., 2007).

It is impossible to eradicate infections with fungicides without injuring the fruit excessively. The penetrating power of gamma-rays is more than fungicides. These rays reach decay organisms in aereas of fruits not accessible to chemicals (Tiryaki, 1990). The advantage of gamma radiation is the high penetrability and uniformity of the dose, which permits to treat products of different sizes and shapes (Jarrett, 1982).

Disadvantages of irradiation treatment

The process of irradiation essentially adds a small amount of energy to food. As such, many radiolytic products are generated, but in very small numbers. Heat processing forms the same general types of molecules, but in larger numbers, because the amount of energy added to foods is often greater than with irradiation. Induction of radiation-resistant microbial populations occurs when cultures are experimentally exposed to repeated cycles of radiation. Mutations in bacteria and other organisms develop with any form of food processing, including ionizing radiation, heat, drying, and ultraviolet light. Radiation does not produce mutations by unique mechanisms. Further, mutations from any cause can result in greater, less, or similar levels of virulence or pathogenicity from parent organisms. Although it remains a theoretical risk, several international reviews cite no reports of the induction of novel pathogens attributable to food irradiation. Similar concerns exist about mycotoxins. Experimental data are conflicting, but some studies show an increase in mycotoxin formation after irradiation. One theory is that the higher radio-resistance of molds and yeasts compared with bacteria results in a loss of competitive inhibition of mold and yeast growth. Any mold surviving under treatment with irradiation may be expected to grow more rapidly in the absence of competitors and eventually dominate the mycoflora. In the absence of temperature abuse in storage, the available evidence indicates that treating products with ionizing energy does not add to that hazard. More nutrients are made available for fungi by irradiation. This is an area in which additional study would be useful (Shea et al., 2000).

Palou et al. (2007) did not observe any resistance to green and blue molds on mandarins exposed to X-irradiation at doses from 195 to 875 Gy. Contrarily, green mold development was slightly favored in fruit treated at 875 Gy when *P. digitatum* was inoculated 6 days after irradiation. This might be related to a negative effect of X-rays at this dose on the physical and/or physiological condition of the fruit rind that would facilitate the fungal mycelial growth through the albedo and flavedo cells. The negative effect may include the induction of some incipient peel damage that was not readily visible.UV-C irradiation was not able to reduce the occurrence of *Colletotrichum gloeosporioides* lesions and caused browning in papaya fruit (Cia et al., 2007). Similar findings have been reported earlier indicating that smaller doses of UV-C reduced the development of *B. cinerea* in

Table 4. Lethal gamma radiation doses to young growing mycelium of some fungi (Beraha et all., 1960).

Organism	Source	Dose (*10^5 rad) on		
		Tochinai	Czapek	Host
Phytophthora infestans	Potato	0.25	0.25	0.25
Phomopsisi citri	Orange	0.44-0.96	0.44-0.96	0.91-1.45
Penicillium digitatum	Lemon	1.10-1.48	0.44-0.94	1.82-2.10
Penicillium italicum	Orange	1.43-1.47	1.19-1.43	1.57-1.82
Penicillium expansum	Apple	1.35-1.40	1.95-2.52	1.82-2.74
Botrytis cinerea	Grape, strawberry	0.95-1.86	0.95-1.86	2.74-4.56
Monilinia fructicola	Peach	1.38-1.85	0.90-1.38	1.37-1.82
Sclerotinia sclerotiorum	Bean	1.73-2.13	1.73-2.13	2.28-2.73
Rhizopus nigricans	Peach	2.74-3.52	3.52-4.43	1.82-2.28
Alternaria tenuis	Tomato	4.20-4.57	4.20-4.57	2.74-4.56

table grapes, but caused fruit browning (Camili et al., 2004).

Mechanism of action of irradiation on micro-organism

The content of the major phenolic compounds present in the peel of clementine mandarins significantly increased on fruit that had been previously irradiated with gamma-rays at 300 Gy. This increase was correlated with an enhancement of the activity of the enzyme phenylalanine ammonia-lyase (PAL). Ionizing radiation can stimulate the biosynthesis of constitutive and/or induced phenolic compounds that could extend storage life and in some cases induce fruit resistance against pathogens (Palou et al., 2007). In their study, if such bioactive compounds were actually synthesized, it was at levels not high enough to effectively induce disease resistance under our experimental conditions and the synthesis was not influenced by either X-ray dose, time between irradiation and pathogen inoculation, or incubation time after inoculation. They suggest, therefore, that the direct effects of irradiation on the fungal structures growing in the rind were more important for disease reduction than a possible indirect effect on the fruit mechanisms of defense. This assumption is further supported by the fact that X-irradiation considerably inhibited the sporulation of both *P. digitatum* and *P. italicum* on decayed mandarins.

A number of researchers indicated that gamma-rays inhibited fungal development and mycotoxin production during the food storage. The effect of irradiation depends on fungus type, application dose, moisture content and compostion of food, and storage conditions (Aziz et al., 2006; Kabak and Var, 2005). The effect is also depends on environmental factors such as, composition and moisture content of irradiated medium, temperature and presence of oxygen during the irradiation, being fresh or frozen (Smith and Pillai, 2004). In another explanation, surviving of microbial cells depends on the resisitence and recovery status of cell, irradiation dose, pH, atmospheric conditions and chemical composition of food (Monk et al., 1995).

Low irradiaton doses (for example 1 kGy) stimulated fungal development for both *invitro* and *in vivo* studies (Tiryaki, 1990). After 40 days irradiation, lesion diameters were 36.21 and 34.75 mm for 1 kGy and control treatment, respectively, in Ankara pears inoculated with *Penicillium expansum*. This supports stimulative effect of low gamma-rays (Tiryaki and Maden, 1991). Similarly 1 kGy of gamma irradiaton stimulated aflatoxin occurence. Whereas 3 to 4 kGy inhibited fungal and mycotoxin development (Kabak and Var, 2005).

Lethal gamma radiation doses required for pathogens in the host (*in vivo*) are higher than in the culture (*invitro*) media (Table 4) (Beraha et al., 1960). Irradiation dose rate is also important for inhibition of fungal development. Beraha (1964) worked on the effect of dose rate and demonstrated that high dose rate was more effective than low dose rate. *B. cinerea* infection was inhibited with the 125 to 150 krad of irradiation, at the dose rate of 25 krad/min; whereas, infection was not inhibited with the 200 krad at the 2.5 krad/min.

Irradiation kills microbes primarily by fragmenting DNA. The sensitivity of organisms increases with the complexity of the organism. Thus, viruses are most resistant to destruction by irradiation, and insects and parasites are most sensitive. Spores and cysts are quite resistant to the effects of irradiation, because they contain little DNA and are in highly stable resting states. Toxins and prions, which have few chemical bonds to disrupt, are resistant to irradiation. The conditions under which irradiation takes place (that is, temperature, humidity, and atmospheric content) can affect the dose required to achieve the food processing goal, but these are well-described and easily controlled (Shea et al., 2000).

Tiryaki et al. (1994) worked on pathogenicty of irradiated fungi. Effect of irradiation on pathogenicity cultural charasteristic and sporulation of fungi have been

Table 5. Effects of postharvest UV-C treatment on *B. cinerea* disease for freesia inflorescences (Darras et al., 2010).

Factor	Disease parameter		
	Disease severity (score 0-4)	Lesion number	Lesion diameter (mm)
(1) UV-C irradiation*			
Before inoculation	3.1[b]	83[b]	0.91[b]
After inoculation	1.3[a]	36[a]	0.79[a]
(2) UV-C doses (D) (kJ m^{-2})			
0.0	2.9[d]	75[c]	0.87[b]
0.5	1.8[b]	49[ab]	0.79[a]
1.0	1.5[a]	43[a]	0.80[a]
2.5	1.9[b]	52[b]	0.85[ab]
5.0	2.1[c]	55[b]	0.88[b]
(3) Interaction I×D			
	P < 0.05	P < 0.05	P < 0.05

*Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

investigated by reisolation. It was found that there were no differences at these properties of fungi between irradiated and unirradiated samples. The gamma irradiation dose which inhibits decay was determined in apple, quince, and peach inoculated with *P. expansum*, *Monilinia fructigena* and *Rhizopus stolonifer*, respectively. Doses of 1, 2, 3 and 3.5 kGy did not inhibit decay on fruit, but infection was delayed for a certain period.

Time between inoculation and irradiation

Time interval between inoculation and irradiation affected the growth response of *Monilinia fructicola* infections and the irradiation dose needed for it control. When "firm-ripe"peaches were irradiated (200 krad) within 24 h after inoculation, only 10% of the inoculations formed lesions. Postponing irradiations to 36, 48 or 60 h after inoculation increased the incidence of lesion to 60, 80 and 90%, respectively (Kuhn et all., 1968).

A factor that could adversely influence the effectiveness of the treatments was the extended period of time between inoculation and irradiation (about 38 h at about 20°C). According to Spalding and Reeder (1985) the incidence of green mold was lower on grapefruit irradiated with gamma-rays 2 h after artificial inoculation with *P. digitatum* than on fruit irradiated 24 to 72 h after inoculation.

Moreover, it was observed that irradiation was more effective against citrus postharvest diseases when applied before extensive fungal development.

Nevertheless, satisfactory commercial control of citrus *Penicillium* decay usually requires the effective control of infections that were initiated in the field at least 24 h before the application of the antifungal treatment (Palou et al., 2007).

Irradiation before and after inoculation

Darras et al. (2010) searched germicidal and inducible host defense effects of UV-C irradiation on petal specking caused by *B. cinerea*. UV-C irradiation of freesia inflorescences after artificial inoculation with *B. cinerea* was more effective in reducing petal specking, compared to UV-C treatment before artificial inoculation. Cut freesia inflorescences exposed to 1 kJ m^{-2} UV-C after artificial inoculation with 10^4 conidia ml^{-1} displayed reduced disease severity scores, lesion numbers and lesion diameters by 74, 68 and 14%, respectively, compared to non-irradiated inflorescences. In contrast, UV-C irradiation with 1 kJ m^{-2} before artificial inoculation reduced lesion numbers and lesion diameters by 13 and 24%, compared to the non-irradiated controls. Higher UV-C doses of 2.5 or 5 kJ m^{-2} reduced disease severity scores, lesion numbers and lesion diameters when applied after artificial inoculation, but enhanced disease when applied before artificial inoculation.

Inflorescence irradiation following artificial inoculation with *B. cinerea* generally conferred significant (P < 0.05) disease reduction compared to irradiation prior to artificial inoculation (Table 5). Disease severity scores and lesion numbers on inflorescences irradiated after artificial inoculation was significantly (P < 0.05) lower than the ones irradiated prior to artificial inoculation at all UV-C doses tested. Irradiation of inflorescences with 0.5, 1, 2.5 or 5 kJ m^{-2} UV-C after inoculation reduced disease severity scores and lesion numbers by 44, 70, 74, and 59% and by 37, 62, 68 and 60%, respectively (Figure 1). UV-C irradiation suppressed petal specking caused by *B. cinerea* when applied after artificial inoculation (Darras et al., 2010). In a research carried out by Palou et al. (2007), irradiation performed before and after inoculation was evaluated with respect to fungal inhibition. There was

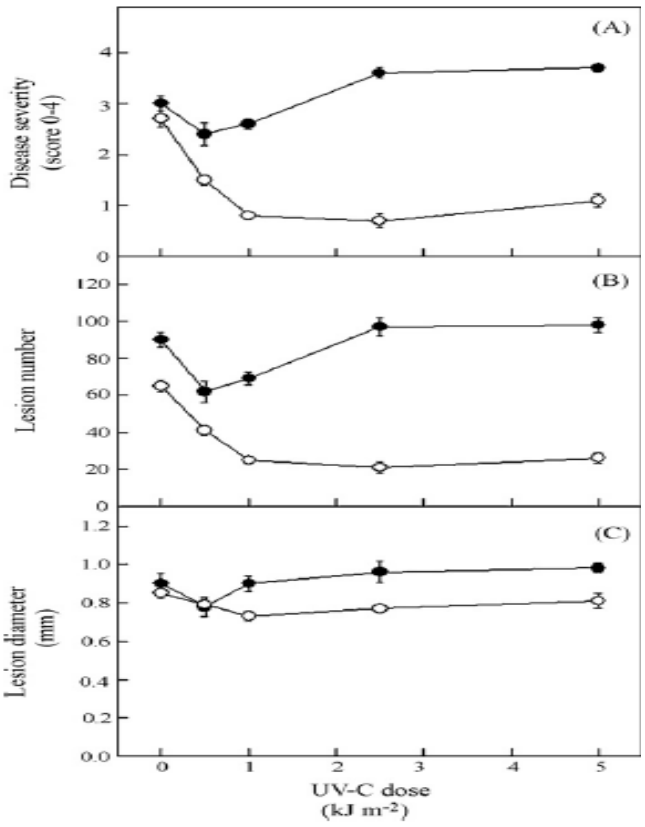

Figure 1. Disease severity scores (A), lesion numbers (B) lesion diameters (C) on freesia irradiated with UV-C (Darras, et al., 2010).

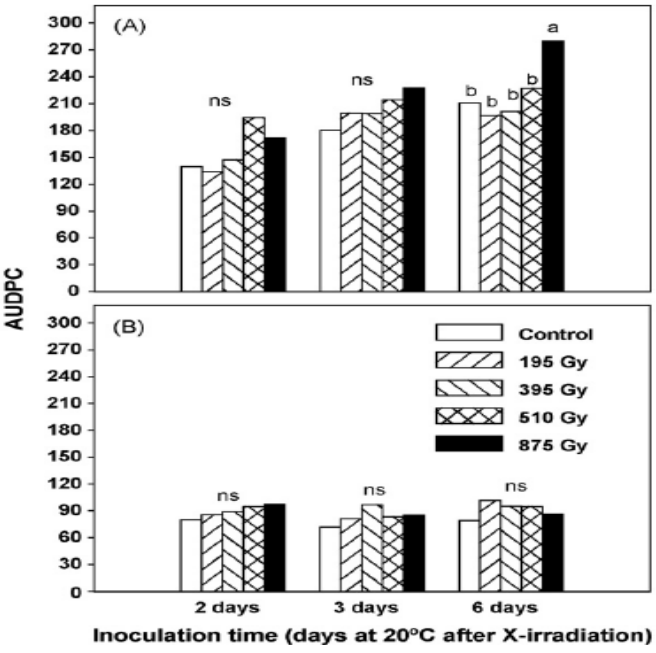

Figure 2. Area under the disease progress curve (AUDPC) of *P. digitatum* (A) and *P. italicum* (B) on clementine mandarins irradiated with X-rays (Palou et al., 2007).

not any difference between control fruit samples and treated fruit samples (that is, inoculated after irradiation) with respect to disease severity and disease incidence. It can be concluded that gamma and UV-C (254 nm) irradiations are physical treatments, entire effect on fungi occur during irradiation process.

Induction of fruit disease resistance by irradiation

Palou et al. (2007) carried out a detailed research about induction of fruit resistance to disease by X-ray irradiation. Non-inoculated mandarins that had been irradiated with X-rays at 0 (control), 195, 395, 510 and 875 Gy were kept at 20°C for 2, 3 or 6 days. After each of these time periods, irradiated fruit were inoculated with 10^5 spores' ml^{-1} of *P. digitatum* or *P. italicum* and incubated at 20°C for 7 days. Each pathogen was inoculated on different sets of fruit. For each pathogen, irradiation dose and inoculation time, four replicates of 10 fruits were used. Disease incidence and severity were evaluated after 3, 5 and 7 days of incubation. Irrespective of the X-irradiation dose and the time that inoculated fruit were incubated at 20°C (3, 5 or 7 days), neither the incidence (Figure 2) nor the severity (Figure 3) of both green and blue molds on artificially inoculated mandarins were significantly affected by exposure to X-rays. Significant differences among treatments were only observed for AUDPC on clementines inoculated with *P. digitatum* 6 days after irradiation; AUDPC was significantly higher on fruit treated at 875 Gy than on control fruit or fruit irradiated with other doses. Therefore, under experimental conditions, fruit resistance to disease not only was not increased but also was reduced by X-irradiation. Therefore, X-ray treatment did not induce disease resistance in the rind of irradiated fruit.

To check the possibility of resistance induction by irradiation, papayas were also inoculated after the treatments. It seems that papaya inoculation 24 h after treatment did not induce resistance since lesion diameter was not reduced. It can also be seen that UV-C did not reduce pathogen sporulation on fruit lesions. Thus, it is possible that fruit inoculation 24 h after the treatments did not stimulate defense responses in the fruit (Cia et al., 2007).

COMBINATION OF IRRADIATION WITH OTHER TREATMENTS AGAINST THE POSTHARVEST DISEASES

Recently, combined treatment is recommended to control the postharvest diseases. The main purpose of combination is to increase the effectiveness, to decrease the negative effect of application by exposure to lower doses compared to single application. The effect of irradiation is more promising when applied in combination

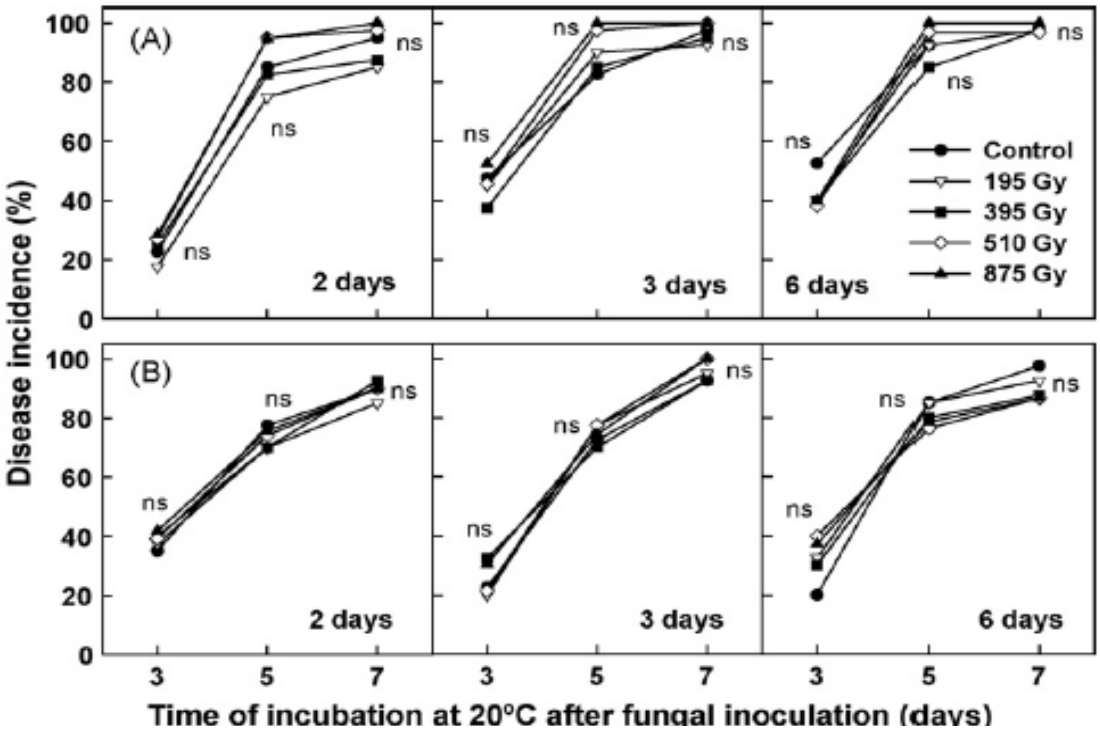

Figure 3. Incidence of *P. digitatum* mold (A) and *Penicillium italicum* mold (B) on clementine mandarins (Palou et al., 2007).

with hot water treatment, chemicals, such as SO_2 fumigation and cold storage treatment (Beraha et al., 1960; Tiryaki et al., 1994). Sodium carbonate (SC) treatment is also another alternative combination against the postharvest diseases.

Consumers are demanding less chemical residue on produce, and many fungi are developing resistance to commonly used fungicides. Since the use of fungicides is becoming more restricted due to health concerns, many alternatives to chemical control have been investigated, but none was able to provide the level of control of synthetic fungicides. While heat treatment virtually eliminates decay if fruit are inoculated prior to heating, it has little effect when infection occurs after heating, therefore, having no protective effect. Likewise, sodium bi-carbonate (SBC) does not provide persistent protection of the fruit from re-infection after treatment. The major limitations with biocontrol are the lack of eradicative activity, and a narrower spectrum of activity than is found with synthetic fungicides. The effect of environmental factors on biological control is also generally greater than the effect of fungicides. A combination of three methods described above may complement one another to overcome the shortcomings of each. Combination of several of these alternatives increases their effectiveness (Conway et al., 2004). But some workers revealed that both UV-C and gamma-rays reduced storage rot, but the combination of UV and gamma showed no advantage over the use of UV or gamma alone (Lu et al., 1993).

Combination with sodium carbonate

Palou et al. (2007) investigated the effects of X-ray irradiation and SC treatments on postharvest *Penicillium* decay in mandarins. As shown in Figure 4, by storage at 20°C, SC treatment with 875 Gy is more effective with respect to disease severity, disease incidence and sporulation of *P. digitatum* and *P. italicum.* Green mold severity as lesion diameter was only significantly reduced by irradiation at the highest dose of 875 Gy. Similarly, blue mold severity was not significantly reduced by SC treatment alone, but it was 25 to 35 mm by treatment at both X-ray doses (Figure 4). Comparable results were obtained with mandarins cold-stored at 5°C for 21 days. For both molds, SC treatment alone did not significantly affect either disease incidence and severity or pathogen sporulation (Figure 5). Incidence of both diseases on previously SC-treated fruit was markedly reduced by X-irradiation at both doses after 7 days of cold storage.

Palou et al. (2007) observed remarkably lower efficiencies of sodium carbonate on citrus postharvest green and blue molds than Smilanick et al. (1999). Factors that could account for this lack of effectiveness in reducing either disease incidence and severity or pathogen sporulation may include: The high initial susceptibility to

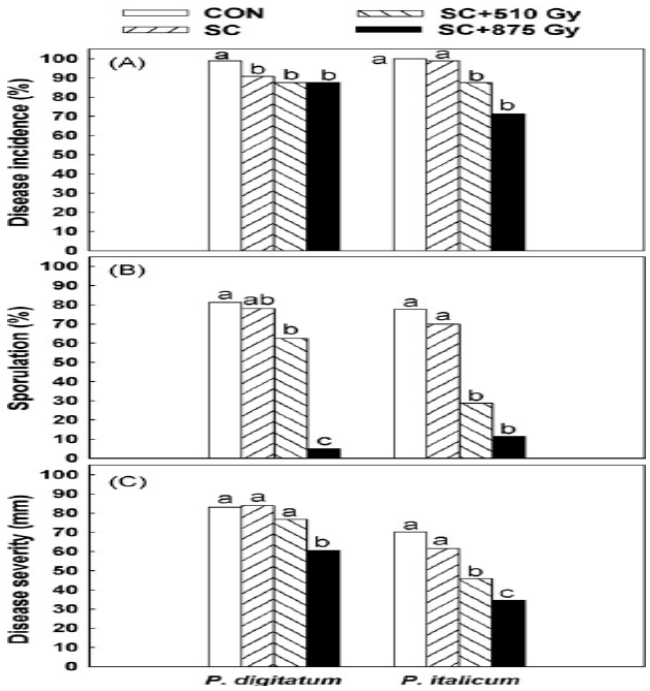

Figure 4. Percentage of infected (A) and sporulated (B) fruit and lesion size (C) on clementine mandarins incubated at 20°C for 7 days (Palou et al., 2007).

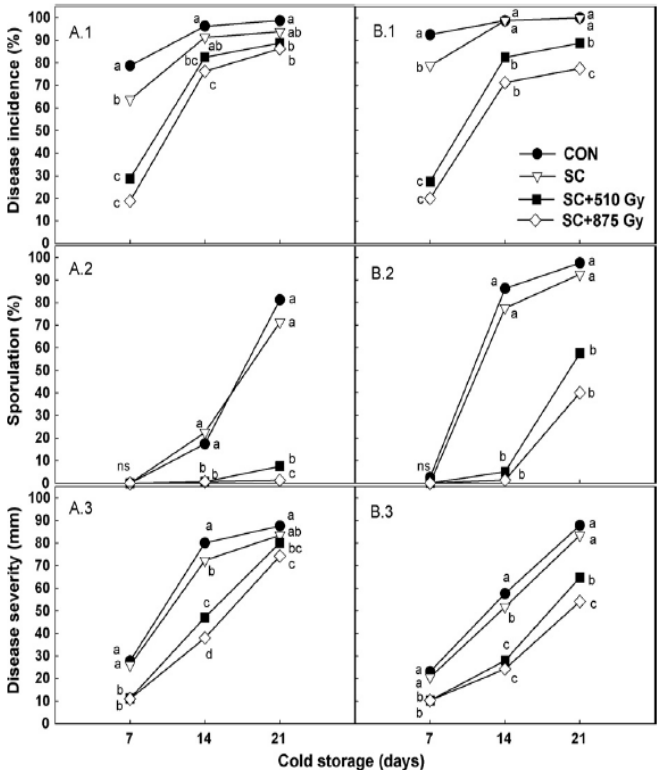

Figure 5. Percentage of infected (1), sporulated (2) fruit and lesion size (3) on clementine mandarins artificially inoculated with *P. digitatum* (A) or *P. italicum* (B) and incubated at 5°C for 21 days (Palou et al., 2007).

decay of the fruit used in the experiments, the use of non-heated SC solutions, and the rinse of treated fruit with tap water. It has been shown that the effects of SC, SBC, and other low-toxicity food additives on *Penicillium*-infected citrus fruit are fungistatic, not very persistent, and highly dependent on the host species and its physical and physiological condition.In contrast to fungal growth, pathogen sporulation was clearly inhibited on inoculated mandarins by the combined treatments (irradiation and SC), especially that of *P. italicum* on fruit incubated at 20°C and that of *P. digitatum* on fruit cold-stored at 5°C. Since SC does not exert anti-sporulant activity, this effect should be attributed to irradiation. The reduction of spore production has commercial value because stored citrus fruit are usually treated with fungicides; if resistance develops among these pathogens, the treatment would reduce the proliferation of resistant spores and presumably would prolong the useful life of postharvest fungicides. Further, *Penicillium* spores that are produced from stored fruit are a significant source of contamination for healthy adjacent fruit, and for packages, walls, and floors of rooms. Thus, irradiation treatment could greatly reduce the load of airborne pathogenic spores (Palou et al., 2007). Results from this work suggest that some technological aspects of the integration of SC and X-ray treatments should be improved for satisfactory control of established infections of *P. digitatum* and *P. italicum* on clementine mandarins. Heating SC solutions, non-rinsing SC-treated fruit, reducing the time between inoculation and irradiation, or even applying first the irradiation then the SC treatments could presumably enhance the effectiveness of the combined treatments.

Carbonic acid salts, such as sodium carbonate (SC, Na_2CO_3, soda ash) and sodium bicarbonate (Palou et al., 2001), are common food additives allowed with no restrictions for many applications. SC has been re-examined during recent years as a potential alternative to synthetic fungicides to manage citrus postharvest diseases because it is inexpensive, readily available, and can be used with a minimal risk of injury to the fruit. In general, carbonic acid salts are considered to be good candidates to be used in combination with other chemical, physical, or biological methods for the integrated control of postharvest diseases (Palou et al., 2002).

Rinsing the fruit at low pressure has been an effective method to avoid potential negative effects (loss of weight and firmness during cold storage) of SC treatments on the quality of clementine mandarins. However, in contrast to what was observed on oranges (Smilanick et al., 1999), rinsing of SC-treated clementines resulted in a significant loss of SC effectiveness against green mold. In order to preserve fruit quality and avoid potential interactions of X-rays with SC residues present on the surface of the mandarins, it was decided to rinse the fruit after SC treatment. This fact could help explain why SC effectiveness was less in this work than in previous

experiments with unrinsed clementines (Palou et al., 2007).

Spores of both *P. digitatum* and *P. italicum* were found in early research to be more sensitive to irradiation than spores of other major citrus postharvest pathogens such as *B. cinerea, Diplodia natalensis, R stolonifer*, or *Alternaria citri* and were killed at very high rates (>95%) with a dose of 1000 Gy. However, effective control of established infections of *Penicillium* on oranges or lemons required irradiation doses higher than 1000 Gy and, in general, such doses induced apparent rind injury.

Therefore, the effectiveness of irradiation treatments alone or the potential synergy between SC and X-ray treatments against *Penicillium* established infections were not evaluated. Instead, the objective was to assess directly the disease control ability of the combined treatments. The integration of SC and X-irradiation, especially at the highest dose of 875 Gy, significantly reduced disease incidence and severity of both green and blue molds on mandarins stored at either 20 or 5°C (Palou et al., 2007).

Combination with heat treatment

A detailed study on radiation-heat synergism for inactivation of market disease fungi of stone fruits was carried out by Sommer et al. (1967). Synergistic effects of combined gamma radiation and heat treatments were compared with the single treatments for inactivation of spores of postharvest pathogens of *Prunus* spp. Interaction between treatments sometimes caused a 5- to 10-fold increase in inactivation.

The amount of synergism and the preferred sequence of application for maximum fungicidal effect depended upon the pathogen. With *R. stolonifer,* the maximum effect occurred if irradiation was first. In all other species studied, the reverse sequence resulted in greatest inactivation. Studies with fruit inoculated with *M. fructicola* demonstrated the advantage of heat sensitization before irradiation in brown rot control.

Hot water reduces decay substantially, but the risk of injury, weight loss, and the lack of antifungal residues has made this treatment a less attractive option than the relative ease of application, efficacy, and persistent protection offered by fungicides (Margosan et al., 1997).

Palou et al. (2007) did not use heated solutions in their experiments because according to previous reports, the combination of hot water with irradiation resulted in detrimental effects on the quality of treated clementines. Furthermore, the integration of hot water and irradiation for the control of green mold on oranges and grapefruit yielded contradictory results in previous research. While in some studies such combination resulted in synergistic effects, in other studies no benefits were observed.

Various non-chemical approaches have been investigated or proposed in recent years. Several studies have shown that hot water treatments have the potential to control postharvest diseases of peaches. In addition, biological control of postharvest diseases of stone fruits has been pursued actively by using bacteria and yeast antagonists (Karabulut and Baykal, 2004).

Heat treatment (38°C for 4 days) was effective in eradicating *P. expansum* Link on apples initially but exhibited no residual activity. The mode of action of the heat treatment seems to be both through direct interactions with the fungus itself, and *via* physiological responses of the fruit tissue. *In vitro* studies showed that both germination and growth declined when fungi were exposed to extended periods at higher temperatures. Heat treatment may also alter the susceptibility of the host to pathogens by the formation of an inhibitory substance in the peel (Conway et al., 2004).

The effect of irradiation is more promising when applied in combination with hot water treatment and chemicals, such as SO_2 fumigation, and cold storage treatment. It is possible to obtain more hopeful result with decreasing the time between irradiation and harvest. Less decay is observed when fruits were irradiated soon after picking than after a storage period (Tiryaki et al., 1994).

Combination with chemicals

Previous study has demonstrated that radiation, heat and chemical is the most efficient combination for the inhibition of postharvest diseases. Benomyl at 1000 ppm was the best single treatment for control of *P. expansum* in apples at about 25°C although it was not completely effective. Double combinations of hot water followed by irradiation and one triple combination of 50°C/10 min with irradiation and aureofungin could provide 80 to 100% control depending on quantity of inoculum. The double combination of 250 ppm benomyl preceded by hot water (50°C/5 min) at an interval of ½ h or the triple combinations of 50°C/10 min -150 krad –irradiation-250 ppm benomyl and 56°C/4 min 150 krad of radiation -1000 ppm aureofungin in these sequences completely controlled the 2-day–old infections of apples during the 3-week holding period at 25 1°C and extended the storage life (Roy, 1975).

Georgiev (1983) worked on combined treatment with irradiation and chemicals against the *B. cinerea* infection in “Bolgar” grapes at 80% relative humudity and 4 to 10°C storage. Captan +1kGy treatment was more effective against the *B. cinerea* infection than 2 and 3 kGy treatment. Similar findings were reported by Shirzad and Langarek (1984). They investigated irradiation and SO_2 treatment to increase shelf-life of grapes.

Combination with modified amosphere packaging

Several studies demonstrated the inhibitory effect of modified atmosphere packing (MAP) on postharvest

Table 6. Lesion diamater (mm) of Ankara pears inoculated with *B. cinerea* (Tiryaki and Maden, 1991).

Dose (kGy)	Day after irradiation			
	10	20	30	35
0	25.63^{A*a**}	35.04^{Aa}	40.58^{Bb}	46.29^{Bb}
1	22.83^{Bb}	34.54^{Aa}	49.46^{Aa}	53.33^{Aa}
2	15.79^{Cc}	25.88^{Bb}	43.58^{Bb}	50.63^{ABab}
3	13.21^{Dd}	20.88^{Cc}	32.13^{Cc}	37.54^{Cc}

* Figures followed by different capital letters differ significantly at p< 0.05; *** figures followed by different lower-case letters differ significantly at p<0.01.

Table 7. Lesion diamater (mm) of Ankara pears inoculated with *P. expansum* (Tiryaki and Maden, 1991).

Dose (kGy)	Day after irradiation			
	10	20	30	40
0	12.13^{A*a**}	19.20^{Aa}	26.29^{Aa}	34.75^{Aa}
1	11.29^{Aa}	17.92^{Aa}	25.63^{Aa}	36.21^{Aa}
2	10.13^{Bb}	14.71^{Bb}	19.21^{Bb}	28.46^{Bb}
3	9.67^{Bb}	12.08^{Bb}	15.71^{Bb}	22.88^{Cc}

* Figures followed by different capital letters differ significantly at p< 0.05; *** figures followed by different lower-case letters differ significantly at p<0.01.

Table 8. Development of rot (lesion diameter, mm) on quince fruits wound-inoculated with *Monilinia fructigena* (Tiryaki et al., 1994).

Dose (kGy)	Day after irradiation				
	10	14	24	31	39
0	34.87^{a*}	44.95^{a}	59.95^{a}	71.15^{a}	81.55^{a}
1	29.35^{a}	40.70^{a}	55.40^{a}	65.70^{ab}	76.35^{ab}
2	20.00^{b}	25.90^{b}	37.90^{b}	49.15^{c}	59.50^{c}
3	28.10^{ab}	36.00^{ab}	45.90^{ab}	55.30^{bc}	62.00^{bc}

* Figures followed by different letters differ significantly at p< 0.05.

pathogens (Karabulut and Baykal, 2004). The effect of combining low-dose irradiation (1.75 kGy) with MAP on the microbiological and sensory quality of pork chops stored at refrigeration temperatures was studied by Grant and Patterson (1991). The microflora of irradiated MAP pork was almost exclusively composed of lactic acid bacteria, predominantly *Lactobacillus* spp. Modified atmospheres containing either 25 or 50% CO_2, balance N_2, resulted in the best microbial control in irradiated pork held at 4°C, compared to an unirradiated MAP control, and these atmospheres were subsequently used in sensory studies. The atmosphere containing 25% CO_2, 75% N_2 maintained the uncooked color and odour of irradiated pork chops more effectively than 50% CO_2, 50% N_2. Therefore packaging in a modified atmosphere containing 25% CO_2, balance N_2, followed by irradiation to a dose of 1.75 kGy is recommended to improve the microbiological and sensory quality of pork chops.

Combination with cold storage

A few workers reported that it is not possible to inhibit postharvest fungi by irradiation. It delays only fungal development at different degrees (fungustatic effect). 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.*

'Golden Delicious' apple fruit inoculated with *Colletotrichum acutatum* did not decay during storage at 0°C for four months, confirming earlier observations with this pathogen. Therefore, fruit were stored at 20°C for an additional two weeks to allow decay to develop so that the effectiveness of the various treatments could be determined. *P. expansum*, however, caused extensive decay, even under cold storage conditions, indicating that it is a much more aggressive pathogen than *C. acutatum* (Conway et al., 2004).

In a study performed by Tiryaki and Maden (1991), required gamma-irradiation dose was determined for the inhibition of infection in Ankara pears inoculated with *P. expansum, B. cinerea* and *Rhizopus nigricans* at the 0°C and 85 to 90% relative humidity. After 10 days treatment, diameter of lesion was 25.63 mm in unirradiated Ankara pears inoculated with *B. cinerea.* Whereas irradiated with 3 kGy dose lesion diameter was 13.21 mm. After 20 days irradiation these values were 20.88 and 35.04 mm for 3 kGy and control samples, respectively (Table 6). As to come, *P. expansum*, after 10 days treatment, lesion diameters were 12.1, 11.29, 10.13 and 9.67 mm in 0, 1, 2 and 3 kGy treated pears, respectively. Ankara pears inoculated with *B. cinerea*. After 40 days irradiation, lesion diameter was 36.21 and 34.75 mm for 1 kGy and control treatment in Ankara pears inoculated with *P. expansum* (Table 7). This supports stimulative effect of low gamma rays.

The gamma irradiation doses inhibiting decay were determined in apple and quince inoculated with *M. fructigena* (Tiryaki et al., 1994). The diameters of rot on quince which were wound-inocolated with *M. fructigena* at each dose level are shown (Table 8). Although the differences between 2 and 3 kGy was not statisticaly significant at P< 0.05, the most inhibitory irradation dose for quince rot after 39 days was 2 kGy. In general, doses of 1, 2, 3 and 3.5 kGy did not inhibit decay on fruit, but infection was delayed for a certain period (Tiryaki et al., 1994).

Combination with biocontrol agent

Biological control is another alternative to chemical control that shows effectiveness in controlling postharvest diseases. The reduction of decay by biological control is

generally more variable than for fungicides since biocontrol is affected more by environmental factors. As fruit mature, higher concentrations of the biocontrol antagonist must be used to achieve the same level of control as on immature fruit (Conway et al., 2005).

D'hallewin et al. (2005) worked on combination of ultraviolet-C irradiation and biocontrol treatments to control decay caused by *P. digitatum* in orange fruit. The combination of the yeast *Candida oleophila* strain '13L' with UV-C irradiation evidenced a synergistic effect in reducing *P. digitatum* mould and only 11% of the artificially inoculated wounds were infected. Adversely, when the bacteria *Bacillus subtilis* strain 'B160' was combined with UV-C irradiation no synergistic effect was achieved. By using only yeast, bacteria or UV-C treatments the decay percentage was reduced by 79.6, 55 and 75%, respectively. The phytoalexin scoparone accumulation was high in all treatments where UV-C was applied but the highest values were found when combined with the yeast. Population growth of bacteria *in vivo* was halved when fruit was irradiated, whereas direct irradiation of bacteria did not affect their growth *in vitro*. An inhibitory effect of the phytoalexin toward the bacteria is suggested as the reason for the growth inhibition *in vivo* when the bacterial treatment was combined with UV-C irradiation.

QUALITY ASSESMENT OF IRRADIATED FRUIT

Beraha et al. (1959) found that textural and skin-color abnormalities (softening and skin browning) were noted following irradiation at 400 000 rep or higher but not at 300 000 rep.

The effect of irradiation doses of 150, 200 and 250 krad on the shelf-life and eating quality of Veteran peaches was studied. These levels did not affect flavor, texture or color as evaluated by a taste panel, but were effective in controlling rot for four weeks (Larmond and Hamilton, 1968).

A study of the effect of gamma irradiation on table grapes has shown that a total dose of 200 krad will increase shelf life by 2 to 3 weeks. This conclusion results from organoleptic and biochemical analyses, and from observation on the colour, flavourand consistency of the grapes, as well as their resistance to attack microorganisms (Donini and Pansolli, 1970).

Previous works demonstrated that it is not possible to kill the post-harvet fungi by irradiation. It delays only fungal development at different degrees (fungustatic effect). Radiation dose required to kill fungi has negative effect in skin colour and texture of stored fruit and vegetables. Therefore, it is important to balance fungal inhibition and organoleptic properties in food irradiation (Beraha et al., 1960; Tiryaki and Maden, 1991).

As stated earlier, rinsing the fruits after SC treatment was an effective method to avoid potential negative effects. But rinsing of SC-treated clementines may result in a significant loss of SC effectiveness against green mold. In order to preserve fruit quality, it was recommended to rinse the fruit after SC treatment (Palou et al., 2007; Smilanick et al., 1999).

In general, the effects of ionizing radiation on the quality of fresh horticultural perishables are affected by factors related to the type of radiation and energy level, the produce itself and the postharvest handling. Although X-irradiation at doses up to 875 Gy followed by either 14 days at 20°C or 60 days at 5°C caused very slight rind pitting, minor decreases in fruit firmness, and modest increases in juice acetaldehyde and ethanol contents, these changes had no practical impact on fruit quality. Rind color, titratable acidity, soluble solids concentration, maturity index and juice yield were not influenced by irradiation. 'Clemenules' can be considered as a clementine cultivar highly tolerant to X-irradiation (Palou et al., 2007).

Physicochemical and orgonoleptic alterations of apple varieties (Golden Delicious, Royal Delicious, Red Delicious and Rich-A-Red) irradiated with 0.1, 0.2, 0.4 and 0.6 kGy and stored at 2 to 4°C for 6 months were investigated and the best results with regard to preservation of organoleptic properties, minimal alteration in texture, amount of total soluble solids, acidity and vitamin-C content were observed in variety Rich-A-Red with 0.1 kGy radiation. It was stated that radiation could be used as an alternative preservation technique for apple varieties (Korel and Orman, 2005).

CONCLUSION

Postharvest losses are very significant issue for storage facilities as indicated above. The lack of an effective postharvest treatment against postharvest decay of fruit highlights the need for developing new control methods (Karabulut and Baykal, 2004). It is therefore necessary to develop alternatives to synthetic chemical control to reduce environmental risks and raise consumer confidence (Conway et al., 2004). Furthermore, concerns about human health risks and protection of the environment associated with fungicide residues, have increased the need for alternatives to fungicide usage (Palou et al., 2007). Although there are various methods to control the postharvest disease, a combination may be needed for better postharvest preservation. In this way, effectiveness of single treatment can be increased; negative impact of each treatment can be minimized by applying low doses of each treatment (Cia et al., 2007).

Food irradiation utilizes a source of ionizing energy that passes through food to destroy harmful bacteria and other organisms. It is capable of improving the safety of many foods, and extending their shelf life.

It is not possible to kill the post-harvet fungi by irradiation. It delays only fungal development at different

degrees (fungistatic effect). Radiation dose required to kill fungi has negative effect in skin colour and texture of stored fruit and vegetables. Therefore, it is important to balance fungal inhibition and organoleptic properties in food irradiation studies (Beraha et al., 1960; Tiryaki and Maden, 1991).

In general, the effects of ionizing radiation on the quality of fresh horticultural perishables are affected by factors related to the type of radiation and energy level, the produce itself and the postharvest handling (Palou et al., 2007).

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