Stewart Postharvest Review 2015, 3:4
www.stewartpostharvest.com
ISSN: 1745-9656

Combination irradiation treatments for food safety and phytosanitary uses

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Purpose of the review: Combination of irradiation treatment with other preservation techniques is of potential importance in enhancing the effectiveness and reducing the energy or dose requirement for destroying food borne illness and spoilage organisms while retaining or improving product quality. Phytosanitary irradiation to control quarantine pests, particularly insects, in traded fresh commodities may also benefit from combination with other disinfestation techniques to enhance effectiveness, and to reduce costs, treatment time, and product damage.
Main findings: Combined preservation treatments can be beneficial to eliminate pathogenic bacteria due to the synergistic or additive effect of the treatments. It also permits less extreme use of a single treatment which may protect the sensory quality of the foods. Combination with modified atmosphere packaging (MAP), refrigeration, freezing or heating has great potential for improving the quality and the safety of fresh and processed foods. Irradiation and heat treatments reduce the numbers of pathogenic bacteria and the level of normal flora, while MAP and cold suppresses the growth of the survivors during subsequent storage. The use of natural antimicrobials at concentrations that do not affect the sensory qualities can increase the relative sensitivity of bacteria by >4-fold and can reduce the radiation dose necessary to eliminate pathogens. Natural antimicrobials are normally not stable over time; microencapsulation and the use of edible coatings can improve stability of antimicrobial formulations and prolong their bioactivity.
Phytosanitary irradiation doses to control insects (50 to 400 Gy) are relatively low compared to doses for food safety and sterilization applications. Lowering doses further could save money on treatment costs by reducing treatment time, increasing the capacity of irradiation facilities, and reducing any problems with commodity quality. Combining irradiation with other insect disinfestation modalities such as cold, heat, fumigation, modified atmospheres, and chemical insecticides is a possible means to reduce the radiation dose, and the duration, level, or concentration of the companion treatment while meeting the technical objectives of the quarantine treatment. Irradiation in combination with cold is particularly promising, as it may be a means to reduce the duration and therefore costs of current cold treatment protocols. Irradiation may also allow use of higher cold temperatures that do not cause chilling injury in cold-sensitive fruits.
Directions for future research: The efficacy of active edible coating and active biodegradable packaging in combination with irradiation for microbial control needs further investigation and should be demonstrated at the industrial level. The use of combined treatments to eliminate viruses and parasites in food is not well studied and should be investigated. The effects of mild heat treatment before irradiation on insect radiotolerance and bacteria radiosensitization should be investigated. The relationship between MAP packaging and insect radiation tolerance needs to be explored for a wider variety of quarantine pest species, including surface pests and inherently tolerant Lepidoptera and mites. Additional research is needed to demonstrate the efficacy of irradiation plus cold combination treatments against insects in fresh produce while assessing commodity quality and the potential for commercial application.

Keywords: irradiation; food safety; phytosanitary treatment; heat; cold; modified atmosphere packaging; bacteria; insects; plant essential oils; bioactive films; quarantine

Abbreviations

CFU	Colony Forming Units
EO	Essential Oil
MAP	Modified Atmosphere Packaging
OR	Oleoresins
RTE	Ready-to-eat
RSS	Relative Radiation Sensitivity

Irradiation for food safety and preservation

Several food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of food poisoning. Current trends towards improvement in safety, quality and convenience of foods, and saving energy in food processing and distribution are increasing interest in developing new combinations of methods for food preservation. Combination of irradiation treatment with other preservative agents seems to be of potential importance in enhancing the effectiveness and reducing the energy or dose

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doi:10.2212/spr.2015.3.4

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requirement for food preservation while retaining or improving product quality.

One of the most important properties of irradiation is to inactivate microorganisms, especially pathogens. Moreover, this technology can be efficient to delay the ripening of fruits, inhibit germination (eg, onion, garlic) and, under certain conditions, deactivate viruses. The biological effects of irradiation are to create damage in the genetic material of the cell causing a lesion in the DNA or breaking both strands of DNA [1]. The DNA damage caused by irradiation generally has the greatest impact on the viability of cells. Types of damage include chemical changes in the nitrogenous bases, insertions or deletions of nucleotides, single or double strand breaks and the emergence of covalent bonds between nucleotides [2]. In a study by FT-IR on the chemical composition of DNA irradiated with gamma rays, the authors have shown that new covalent bonds were formed within the DNA molecules and also suggest that there is formation of inter-strand bonds similar to the inter-strand dimeric photoproducts caused by UV rays[2]. Beauchamp and Lacroix [3] have shown that irradiation can induce dimerization of pyrimidine nucleotides into DNA and demonstrated the higher resistance of *L. monocytogenes* to irradiation than *E. coli* without post-irradiated recovery time. DNA damage prevents multiplication and randomly inhibits cell functions, resulting in the death of the cell.

The sensitivity of microorganisms to irradiation is based on the size of their DNA, the rate at which they can repair damaged DNA and other factors. The size of DNA "target" in the microorganism is one of the most important factors. Parasites and insect pests, which have large amounts of DNA, are rapidly killed by extremely low radiation doses with D_{10} (dose necessary to reduce by one $\log_{10}$ the number of individuals) of 0.1 kGy or less. It takes more irradiation to kill bacteria, because they have smaller DNA with D_{10} in the range of 0.3-0.7 kGy. Some bacteria can form a dense hardy spore, which means they enter a compact and inert hibernation state. Spore forming bacteria are generally the most resistant to irradiation with D_{10} in the order of 2.8 kGy. In addition to direct damage to DNA, indirect action of ionizing radiation on water produces radiolytic products through the reaction of the hydroxyl (OH•) radical. The components of the infected food also affect the bacteria's tolerance to irradiation [4], as well as the efficiency of its DNA repair mechanisms and the environmental conditions present during treatment.

Combined preservation treatments can be beneficial to eliminate pathogenic bacteria due to the synergistic or additive effect of the treatments. It also allows a less extreme use of a single treatment and protection of the sensory quality of the foods [5]. The use of natural antimicrobials at concentration that do not affect the sensory evaluation can increase the bacterial relative sensitivity by more than 4 times and can reduce the radiation dose necessary to eliminate pathogens [6].

Combination with modified atmosphere packaging

Combination with modified atmosphere packaging (MAP), refrigeration, freezing or cooking has great potential for improving the quality and the safety of fresh and processed foods [7, 8, 9]. Irradiation treatment reduces the numbers of pathogenic bacteria and the level of normal flora, while MAP suppresses the growth of the survivors during subsequent storage. Irradiation under MAP acted synergistically in the killing of bacteria in ready-to-use carrots due to the increase of sensitivity to irradiation under MAP conditions [**10]. Lowering the dose rate of irradiation following MAP storage was also effective to protect the color by the protection of the cellular membrane of mushrooms and improved shelf life [11]. Application of antibrowning solution (ascorbic acid 2%, calcium lactate 1%, citric acid 2% and calcium lactate 1%) by vacuum impregnation before irradiation extended the shelf life of sliced white button mushrooms [12]. A combination of irradiation and MAP treatment can delay the bacterial growth rate during storage of mini-peeled carrots [13]. MAP can prevent whitening development during storage of minimally processed vegetables. Severino *et al.* [**14] showed that combined treatment of γ-irradiation, active edible coating and MAP can be also very effective in decreasing the growth of *E. coli* 0157:H7 and *Salmonella* Typhimurium on green beans during storage.

Combination with heat treatment

Processing at high temperatures is often detrimental to product quality as it causes a significant reduction in nutritional value and changes in organoleptic properties [15]. Thus, less severe heat application during processing is desirable. Several studies have demonstrated the usefulness of mild heat treatment prior to low-dose irradiation in extending the shelf-life of certain fresh fruits and cereal products without affecting their normal quality attribute [16]. Heating and irradiation can work in synergy to increase the shelf life of food, and heating before irradiation can enhance the antimicrobial effects of irradiation [16].

The usefulness of mild heat treatment prior to low-dose irradiation has been demonstrated for preservation of fruit juices and some other processed fruit products, and for inactivation of toxigenic molds on nuts, dried fruits, cocoa beans and maize.

The dose of irradiation needed to control fungal spoilage can result in undesirable changes such as tissue or skin damage, or changes in flavor or texture, and can affect the appearance and normal ripening of the fruit [**17]. The tolerance to irradiation varies among species and varieties and is influenced by ripeness at the time of treatment [18, 19]. In the case of fungi, heat treatment preceding irradiation usually results in a greater antimicrobial effect of all combinations [20]. The use of irradiation in combination with heat also has synergistic effects for deactivation of vegetative bacteria [16] and bacterial spores [21]. Gamma irradiation of *Clostridium sporogenes* and *S.* Typhimurium with sub-lethal radiation doses increases their sensitivity to heat treatment, and the degree of sensitivity increases with irradiation dose [22]. It has been postulated that when irradiation is applied under anoxic conditions, an enhanced killing is observed during heating treatment. According to Kim and Thayer [23] irradiation induces DNA damage whereas heat induces membrane destabilization. With bacterial spores, the effect of heating followed by irradiation seemed to be additive or only slightly more than additive, while the reverse order of treatment (irradiation followed by heating) was found to be synergistic [24]. With fresh fruits and vegetables, it is important to establish the exact parameters such as proper maturity stage, proper pre- and post- treatments, and transport conditions yielding optimum results. For example, in South Africa, the combination of hot water dipping at 55°C for 5 min for mangoes, 50°C for 10 min for papayas, and waxing and irradiation (0.75 kGy) combined with low temperature storage and shipment (7-11°C) have been shown to be particularly effective in controlling fungal and insect attack and in delaying senescence [25]. Transportation trials from South Africa to Europe demonstrated that combination-treated mangoes and papayas may be transported long distances with much lower losses in quality than untreated lots [26]. The combined treatment of mild heat and low-dose irradiation also offers possibilities for delayed ripening and reduction of microbial spoilage of tomatoes, mangoes, peach, papaya and other fruits and vegetables [27, 28, **29, 17, 30]. The causal fungus of anthracnose was sensitive to hot water, and dipping for 5 minutes at 55°C could be sufficient to control this disease. For example in mangoes, the percentage of rotted fruits increased more slowly and the development of ripe skin color was delayed for hot water dipped plus irradiation as compared to the control and irradiated only fruit [17, 30].

Litchi has a very short life due to the bacterial growth and browning reaction caused by degradation of polyphenols; a sequential dip treatment with sodium hypochlorite (0.2%, 4 min, 52°C), potassium metasulfite (3%, 30 min, 26°C) and hydrochloric acid (0.25 N) containing ascorbic acid (2%, 10 min, 26°C) followed by gamma irradiation, helped in overcoming this problem [31]. Dipping of fruits in calcium chloride and irradiation can also have a beneficial effect on the fruit quality. Hussain *et al.* [32] demonstrated that dipping apple in calcium chloride solution (2%) and irradiation at 0.4 kGy resulted in a 4 log reduction in yeast and mold and showed a significant retention of the firmness, juice yield and ascorbic content in fruits.

Enzyme inactivation is an important step to keep the physicochemical and sensory quality of foods during storage of shelf stable foods. Enzyme inactivation is radiation dose dependent, however, heating before irradiation can assure enzyme inactivation. Enzyme inactivation can be achieved by mild treatment at 77'C before irradiation treatment. Heating before irradiation can also decrease the dose needed for food sterilization [33].

Heat resistance of bacterial spores is an important food safety issue. The use of heat treatment or the addition of salts before irradiation can increase the sensitivity of bacterial spores during the irradiation treatment. The beneficial effects of heat treatment and irradiation on *Clostridium sporogenes* in canned luncheon meat and sausage have been explored by Farkas and Andrassy [34] and Farkas *et al.* [35].

Combination with natural antimicrobials

Bacteriocins and essential oils (EOs) are well known as natural antimicrobials. According to Burt [36], out of 3,000 EOs which are already recognized, 300 EOs are commercially important, and EO compounds are generally recognized as safe (GRAS) [37]. Bacteriocins are small peptides produced by lactic acid bacteria; nisin is one of the most common bacteriocins used in the food industry and the only one legally recognized as a food preservative [38]. Nisin is produced by *Lactococcus lactis*, and has demonstrated effectiveness against *L. monocytogenes*, *B. cereus* and other gram-positive bacteria [39].

Dussault *et al.* [**40] evaluated more than sixty-seven commercial EOs and oleoresins (ORs) to determine their antimicrobial activity against six food pathogens and spoilage bacteria *in vitro.* This study showed that allylisothiocyanate, cinnamon Chinese cassia, cinnamon OR, oregano and red thyme all possess high antimicrobial activity against the bacteria tested (gram positive bacteria: *S. aureus, L. monocytogenes*, and *B. cereus* and gram negative bacteria: *E. coli, S.* Typhimurium, and *P. aeruginosa*). The authors also found that citral, coriander, garlic, lemongrass and rosemary were most active against only gram-positive bacteria; whereas cinnamon ceylan, clove, laurel, pimento and winter savory showed high antimicrobial activity against gram-positive and gram-negative bacteria.

The use of different EOs in combination with nisin or pediocin was evaluated by Turgis *et al.* [**41]. The authors observed synergistic or additive effects of γ-irradiation and antimicrobial formulations based on EO with bacteriocin (nisin or pediocin) against several food pathogens. Combination of nisin with *Origanum vulgare* EO showed a synergistic effect on the elimination of *L. monocytogenes* whereas the combination of nisin with *Thymus vulgaris* EO caused a synergistic effect against *S.* Typhimurium. Pediocin allowed a synergistic effect against *Escherichia coli* O157: H7 when combined with *Satureja montagna* EO.

Caillet *et al.* [42] studied the mechanism of action of EOs and irradiation on bacteria murein composition. They found for example that oregano EO and irradiation affected the internal ATP concentration and the murein wall of *S. aureus.* The study showed also that the composition and the relative percentage of various muropeptides present in murein were greatly modified in the presence of EOs, resulting in major effects on the physical integrity of the cell wall. These modifications seem also to be dependent of the radiation dose applied to the bacterial cell or the EO concentration used. There was a significant correlation ($P \leq 0.05$) between the reduction of intracellular ATP and increase in extracellular ATP after treatment of the cells with oregano oil or irradiation.

It has been demonstrated that the active compounds present in natural antimicrobials can improve significantly the radiosensitivity of *L. monocytogenes* when applied on ready-to-use carrots before irradiation treatments [43, 44]. Ndoti-Nembe *et al.* [45] demonstrated that carrots treated with carvacrol with nisin or mountain savory with nisin and then irradiating coated carrots at 1 kGy could reduce *S.* Typhimurium to undetectable level during storage period. The same authors demonstrated that this combined treatment has the capacity to significantly reduce the required irradiation dose to eliminate *S.* Typhimurium from ready-to-eat (RTE) carrots. Application of carvacrol plus nisin or of mountain savory plus nisin on carrots and combination with irradiation at 1 kGy was able to reduce *S.* Typhimurium to undetectable levels during the storage period at 4°C. The combination of carvacrol and nisin also significantly enhance the radiation sensitivity of *B. cereus* since lower D_{10} values were recorded after both single and repetitive irradiation treatments [46].

Tawema *et al.* [47] evaluated the effect of irradiation treatments (0.5 and 1 kGy) combined with small amounts of natural antimicrobials to inhibit the growth of *L. monocytogenes, E. coli*

O157:H7, and total yeasts and molds on RTE cauliflower. In this experiment, the application of irradiation before spraying of natural antimicrobials provided better long-term effectiveness of the combined treatment. Results showed that the combined treatments with natural antimicrobials reduced *L. monocytogenes* as well as *E. coli* O157:H7 below the detection limits for 14 days of storage, while the yeasts and molds were also significantly inhibited. According to El-Fouly *et al.* [48], combined treatments of natural preservatives (organic acids and bacteriocins and EOs) and irradiation can decrease the dose needed to eliminate microorganisms in juice. For example, a dose of 7 kGy is needed to eliminate *Debaryomytces*, but the addition of an antimicrobial formulation based on citric acid, cinnamon and lactic acid was able to eliminate this microorganism at a dose of only 3 kGy.

Dussault *et al.* [49] evaluated the antimicrobial properties of commercial EOs, ORs and pure compounds against six food pathogens. Results showed that allylisothiocyanate, cinnamon Chinese cassia, cinnamon OR, oregano and red thyme showed high antimicrobial activity against all tested pathogens evaluated. Further analysis examined the effect of these selected EOs on controlling the growth rate of mixed cultures of *L. monocytogenes* in ham. A reduction of the growth rate by 19% and 10% was observed when oregano and cinnamon cassia EOs respectively were added in ham at a concentration of 500 ppm. Nisin and oregano and cinnamon EOs used in combination with irradiation inhibited the growth of *L. monocytogenes* on RTE meat [50, **51, 52].

Minced meat (5% fat) inoculated with *Bacillus cereus* spores (10^5 to 10^6 CFU/g) was treated with five EO constituents (cinnamaldehyde, DL-menthol, eugenol, thymol, thymus) [53]. These compounds were sprayed separately onto the meat at concentrations from 0-3% w/w in order to determine the concentration needed to reduce by 1 log the population from activated spores of *B. cereus* ATCC 7004. The best antimicrobial compound selected (*ie*, cinnamaldehyde (1.47% w/w)) was mixed with ascorbic acid (0.5%, w/w) and/or sodium pyrophosphate decahydrate (0.1%, w/w) and tested for its efficiency in increasing the relative radiosensitivity of *B. cereus* spores in minced meat. Irradiation treatment in the presence of the cinnamaldehyde and sodium phosphate decahydrate increased the relative radiation sensitivity of *B. cereus* spores by two fold. The combined treatment produced a substantial reduction of *B. cereus* count during commercial storage (5 ± 1°C) as compared to the treatment of irradiation alone.

Dussault *et al.* [54] evaluated the effect of addition of fermented dextrose containing bacteriocins to pork sausages before irradiation treatment. They found that irradiation treatment alone was able to reduce the initial psycrophilic and mesophilic bacteria by more than 2 log CFU/g and kept the *Lactobacillus* population under the detection limit (100 CFU/g). Results also showed that the fermented dextrose alone was able to extend the shelf life of the sausages from 5 days up to 13 days. At day 13, the sausages treated with fermented dextrose or irradiation alone showed 2 log CFU/g less mesophilic bacteria than the control. When combining irradiation treatment with fermented dextrose another reduction of the microbial count of 1 log CFU/g was observed. When combining the irradiation treatment with the fermented dextrose results showed a reduced growth rate of the psycrophilic and mesophilic bacteria compared to both treatments alone. This study demonstrated that fermented dextrose in combination with low dose irradiation act in synergy to reduce the multiplication of total bacterial flora in fresh sausages.

The risk of *Listeriosis* associated with RTE foods like sausage is also a major concern. Turgis *et al.* [**55] evaluated the synergistic effect of nisin a bacteriocin with irradiation treatment. They found a 1.6 fold increase in the relative sensitivity of *L. monocytogenes* when the sausage was irradiated in presence of the bacteriocin. Zahran and Hendy [56] found that the addition of citric acid before irradiation can reduce significantly the level of *B. cereus* and *S. aureus* in sausage.

Pork jerky is a dried food product, and according to Kang *et al.* [57] it may carry several pathogens. In their study, the addition of leek extract and irradiation treatment (3 kGy) completely eliminated the presence of *E. coli*, *L. monocytogenes* and *S.* Typhimurium.

Turgis *et al.* [58] tested more than 40 EOs on the radiosensitivity of *Salmonella* Typhi inoculated in ground beef and found that spice EOs could be used as effective radiosensitizers to inhibit *S.* Typhi growth in medium fat ground beef. Trans-cinnamaldehyde, clove EO and Chinese cinnamon EO were the most effective to improve radiosensitivity of *S.* Typhi showing a relative radiation sensitivity (RRS = D_{10} control sample/ D_{10} treated sample) of >4.0. Mohamed *et al.* [52] also observed that the combination of natural herbal extracts and irradiation of ground beef lowered TBARS values and off-odor scores and significantly increase the color and acceptability score of the samples.

The use of EOs under MAP conditions may act synergistically to increase the radiosensitivity of bacteria. Turgis *et al.* [59] found that *S.* Typhi was more sensitive under MAP conditions (O_2: 60%, CO_2: 30% and N_2: 10%) than *E. coli.* For example, in presence of oregano, the RRS of *S.* Typhi increased from 3 to 5 when irradiated under MAP as compared to package under air. For *E.coli*, the RRS increased from 3 to 4.4 in the presence of oregano and when packed under MAP as compared to air.

Antifungal effects can also be achieved by combining a treatment of basil EOs and irradiation against molds. Hossain *et al.* [**60] showed that basil EO in conjunction with ionizing radiation controlled the growth of *Aspergillus niger* and *Penicillium chrysogenum* in rice grains. Moreover, the combined treatment of γ-radiation and basil EO increased significantly ($P \leq 0.05$) the RRS to 1.4 and 1.6 for *A. niger* and *P. chrysogenum* respectively, and showed a more pronounced inhibition of fungal growth as compared to individual treatments. The combined treatments showed a promising approach to control food contamination by fungi in ambient storage conditions.

Yun *et al.* [61] demonstrated that the addition of particular food ingredients can increase the efficiency of radiation sterilization; however, bacterial radiosensitization depends on the species of microorganism as well as the form of the food ingredients. Four food ingredients (garlic, leek, onion, and ginger) prepared by freeze-drying, pressurization or ethanol extraction, were added at concentrations of 1% and 5% (w/w) into ground pork and inoculated with *E. coli* or *L. monocytogenes* (10^6 CFU/mL) before irradiation in order to evaluate the bacterial radiosensitization [61]. For *E.coli* inoculated pork, the most efficient ingredient was ethanol extracted leek (RSS = 3.89), followed by freeze dried ginger and freeze dried leek (RRS = 3.66 and 3.63 respectively). The RRS for *L. monocytogenes* was generally lower, and the most efficient materials were pressurized and freeze-dried onion (RRS = 2.13 and 2.08 respectively). This study also showed that an increase of the concentration of the food ingredients did not increase more the bacterial RRS.

Combination with edible coating and encapsulation

Even if natural antimicrobials have good efficiency to reduce the microbial count and can act in synergy during irradiation treatment, the efficiency should be demonstrated during time in storage. Natural antimicrobials are normally not stable over time and the use of encapsulation technologies can help to prolong the bioactivity. Microencapsulation of the antimicrobial formulations was done to verify the potential effect of the polymer to protect the antimicrobial efficiency during storage. Then, combined treatments of antimicrobial formulation with *γ* -irradiation were done to verify the synergistic effect against *L. monocytogenes*. Microencapsulation of EOs-nisin and *γ*-irradiation treatment in combination showed synergistic antimicrobial effect during storage on RTE ham [**51]. Microencapsulated cinnamon and nisin in combination with *γ*-irradiation (at 1.5 kGy) showed 0.03 ln CFU/g/day growth rate of *L. monocytogenes* whereas the growth rate of non-microencapsulated cinnamon and nisin in combination with *γ*-irradiation was 0.17 ln CFU/g/day. Microencapsulation significantly ($P \leq 0.05$) improved the radiosensitivity of *L. monocytogenes*. Microencapsulated oregano and cinnamon EO in combination with nisin showed also the highest bacterial radiosensitization 2.89 and 5, respectively, compared to the control. In this study, microencapsulation of EOs and nisin showed a synergistic anti-listerial effect with γ-irradiation on RTE meat products. This study confirmed that irradiation of ham containing encapsulated cinnamon with nisin showed a strong inhibitory effect up to 28 days and the bacterial count was below detection level. According to the authors, microencapsulation technology with irradiation could be an advanced process to improve the food safety for RTE meats.

Some studies available have demonstrated synergistic effects of coating and irradiation to reduce the content of microorganisms in fruits [62] and vegetables [43, 11]. The combination of an edible coating and irradiation treatment was used to maintain the quality of fresh strawberries. A treatment of 1.5 kGy applied to coated strawberry with a cross-linked edible film [62, 17] was effective in reducing water losses and mold growth and extended the shelf life by over 15 days during storage at 4°C.

A bioactive coating formulation based on modified chitosan containing 0.05% nanoemulsion of mandarin EO was tested in combination with γ irradiation treatment of green bean inoculated with *L. innocua* [**63]. The combined coating and gamma irradiation treatment gave promising results, showing 3.3 log CFU/g initial microbial reduction, and exhibiting a strong synergistic antimicrobial effect. According to the authors, ϒ-irradiation treatments, in combination with the bioactive coating, represent an effective approach to control the growth of *L. innocua* on vegetable foods.

Coating with edible film based on milk proteins could also be efficient in delaying browning by acting as an oxygen barrier or scavenger [64]. Coatings based on milk proteins and irradiation at 1 kGy were also able to protect carrot firmness and dehydration during storage under air conditions [65]. Carboxymethyl cellulose based coating was also able to prolong the shelf life of pear and plum treated at 1.5 kGy [66, 67]. In the case of plum, the combination of irradiation and coating resulted in a 2 log reduction of yeast and mold after one month of storage at room temperature and a better retention of chlorophyll during the first two weeks of storage [66]. Microencapsulation of nisin (63μg/ml) in alginate-crystalline nanocellulose (CNC) showed an increase of the lag phase of bacterial growth up to 28 days in RTE ham [68]. The molecular characterization revealed the interaction between alginate-CNC and nisin which also demonstrated the better retention activity of microencapsulated nisin during storage. The radiosensitization of *L. monocytogens*, *Escherichia coli*, *S.* Typhimurium and aerobic microflora in broccoli florets coated by antimicrobial coatings immobilized in methylcellulose-based coating was evaluated by Takala *et al.* [69]. The coating contained different mixtures of antimicrobial agents such as organic acids/lactic acid bacteria (LAB metabolites), organic acids/citrus extract, organic acids/citrus extract/spice mixture and organic acids/rosemary extract, and coated florets were treated with different doses of γ-irradiation. This study showed that the radiosensitization of bacteria varied according to the bioactive coating that was applied. The antimicrobial coating containing organic acids/LAB metabolites was the most efficient formulation in increasing the radiosensitization (by 2.4 times) of *S.* Typhimurium. A lower relative radiosensitivity (< 1.5) was observed for *L. monocytogenes*; nevertheless the coating formulation used was effective. The antimicrobial formulations used in this study did not affect the sensory attributes of broccoli and represent good potential for industrial applications.

Phytosanitary irradiation

Globalization has resulted in greater trade and transport of agricultural commodities, which has facilitated the introduction and spread of invasive pests to new areas. The establishment of new pests can be costly due to increased crop damage, control programs, and quarantine restrictions on trade. Phytosanitary treatments such as irradiation are applied to fresh agricultural commodities to prevent the introduction and spread of quarantine or regulated pests [**70, **71].

For irradiation, the United States Food and Drug Administration has approved radiation doses up to 1000 Gy (1 kGy) for preservation and disinfestation of fresh fruits and vegetables [72]; this upper limit for phytosanitary irradiation has been adopted by other countries. Disinfestation means controlling any arthropod pests infesting the commodity, particularly insects. Ionizing radiation breaks chemical bonds within DNA and other biomolecules, thereby disrupting normal cellular function in the infesting insect [73]. Irradiation damage is partially caused by the creation of free radicals from oxygen and water which react with and damage nearby molecules. Radiotolerance can vary among the life stages of an insect [74], and between taxonomic groups of insects [75]. The goal of a quarantine treatment is to prevent reproduction, and therefore the required response for a radiation treatment may be prevention of adult emergence [76] induction of adult sterility [77], or F_1 sterility [78].

Table 1: List of generic irradiation doses accepted by New Zealand Ministry for Primary Industries for regulated pests in imported fresh commodities.

Insect taxon[1]	Life stages	Dose (Gy)
Fruit flies (Tephritidae)	Eggs, larvae, pupae	150
House flies (Muscidae)	Eggs, larvae, pupae	60
Leaf miners Agromyzidae)	Eggs, larvae, pupae	100
Other flies (Diptera)	Eggs, larvae, pupae	150
Scale (Coccidae)	All life stages	250
Mealybugs (Pseudococcidae)	All life stages	200
Aphids (Aphididae)	All life stages	250
Beetles (Coleoptera)	Eggs, larvae, pupae	300
Moths (Lepidoptera)	Eggs, larvae	400
Thrips (Thysanoptera)	All life stages	400
Spider mites (Tetranychidae)	All life stages	400
All other mites (Acari)	All life stages	500

Generic irradiation treatments are a major advance that will facilitate worldwide adoption of phytosanitary irradiation Currently, Thailand, Mexico, South Africa, India and Vietnam are exporting irradiated fruit to the U.S [71], and Australia is exporting irradiated fruits and vegetables to New Zealand, United States, Malaysia, and Indonesia [79], using generic treatments. In the U.S., generic doses are 150 Gy for any tephritid fruit fly and 400 Gy for all other insects except the pupa and adult stages of Lepidoptera (moths and butterflies); in New Zealand, generic doses have been established for a wide variety of quarantine pest insect groups, and mites, for the first time (Table 1). Radiation doses to control insects—50 to 400 Gy (0.05-0.4 kGy)—are relatively low compared to doses for food safety and sterilization applications. Yet, lowering doses further could save money on treatment costs by reducing treatment time, could increase the capacity of irradiation facilities, and would reduce any problems with commodity quality. Combining irradiation with other insect disinfestation modalities such as cold, heat, fumigation, modified atmospheres, and chemical insecticides is a possible means to reduce the radiation dose, and the duration, level or concentration of the companion treatment, while meeting the technical objective of the quarantine treatment. No combination phytosanitary treatments using irradiation are approved or in use presently, but possibilities exist. Where research on specific combination treatments for insect pests of fresh produce is scarce, research on stored product pests is presented to illustrate the potential for combined effects.

Combination with cold

Low temperature can be a stressor for insects that reduces survivorship, and cold storage was one of the first methods used for quarantine purposes in traded agricultural commodities [80, 81]. Currently, temperatures of -0.6-2.2°C are used as a quarantine treatment in citrus, table grapes, lychee , longan, and kiwi fruit to control Mediterranean fruit fly and other fruit flies [82, 83]. The use of cold treatment is limited due to the relatively long treatment time (12-24 days) and the sensitivity of many fruits to long periods of insecticidal cold temperatures [84, 85]. An irradiation plus cold storage combination treatment might allow for wider use of cold as a quarantine treatment by reducing the duration of the cold temperature requirement or by allowing the use of slightly higher temperatures that are less damaging to the commodity. Von Windeguth and Gould [86] showed that radiation treatment at 50 Gy followed by cold storage at 1.1°C for 5 days provided quarantine-level security of Caribbean fruit fly, *Anastrepha suspensa*, in grapefruit, but 40 Gy alone also resulted in complete mortality. Irradiation at 30 and subsequent exposure to 2 d at 1.0°C controlled Mediterranean fruit fly larvae in clementine mandarins, whereas 40% of larvae survived to adulthood after 2 d cold exposure without irradiation [87]. Melon fly, *Bactrocera cucurbitae*, and Mediterranean fruit fly third instars irradiated in papaya at 30-35 Gy followed by 11 d at 4°C failed to pupate [88]. Egg hatchability and larval survival of Mediterranean fruit fly in Navel oranges irradiated at 300 Gy was greatly reduced when stored for 7-14 d at 5.5°C compared to storage at 20°C [89]. These results indicate that the radiation dose and the duration of cold can both be reduced when the treatments are used in combination. Additional research is needed to demonstrate the efficacy of irradiation plus cold combination treatment against a variety of quarantine insects in specific commodities under commercial conditions while assessing commodity quality [70]. Cold treatment could gain wider use as a disinfestation treatment if combination with irradiation provides treatment schedules of shorter duration that minimize chilling injury.

Combination with heat

Heat treatments including vapor heat, forced hot air, and hot water immersion have been developed for tephritid fruit fly control in subtropical and tropical fruits such as citrus, papaya and mango [80–91]. Heat-induced mortality is dependent on time and temperature. Heating the commodity before, during or after irradiation may reduce the irradiation dose or the time or temperature requirement for insect control. Research in this area with stored product pests illustrates the concept. Immature stages of the Angoumois grain moth, *Sitrotroga cerealella*, in wheat treated by irradiation (100 Gy) and infrared radiation heating (48,000 BTU/hr IR source, 15 sec treatment at 65 cm distance), showed a 93% reduction in adult emergence, which was greater than predicted by the additive effects of the treatments alone [92]. Similarly, when irradiation was combined with microwave heating (2450 MHz, 25 sec) adult emergence from treated immature stages in Angoumois grain moth was 96% and the combined treatments were synergistic [93]. Effects were synergistic when irradiation was combined with heating from either infrared or microwave radiation for control of the lesser grain borer *Rhizopertha dominica* in wheat [94]. In all these experiments, the results were the same whether the insects were treated by irradiation then heat, or by heat then irradiation. Tilton *et al.* [95] asserted that the dose and therefore the cost of irradiation could be greatly reduced by use of supplemental heat treatments. Microwave or infrared heating might integrate easily into a packing line conveyor system prior to passage through an irradiation chamber [96]. In dried dates, use of a combination treatment consisting of irradiation at 350 Gy and heat treatment at 40°C for 72 h provided satisfactory control of almond moth *Cadra cautella*, and was more effective than irradiation at 700 Gy alone [97]. For fresh produce, heat disinfestation treatments are invariably harmful to commodity quality [84], and therefore irradiation combination treatment holds promise as a means to reduce the required temperatures or treatment duration, and thereby reduce damage.

Combination with plant essential oils

Most research on the pesticidal effects of plant EOs has been conducted with stored product pests [98], although plant pests have also been investigated to a limited extent [99, 100]. The use of the high generic radiation dose of 400 Gy or high concentrations of plant EOs separately may be expensive and time consuming [101, 102]. Exposure to EO vapor during or after irradiation may increase the radio-sensitivity of quarantine pests, allowing for reduced radiation doses and EO concentrations. Adult rice weevil mortality was 25% at 5 d after irradiation at 100 Gy, but increased to 100% with irradiation and continuous exposure to 0.24ul/ml basil oil [103]. Mortality of red flour beetle, *Tribolium castaneum*, treated with a combination of irradiation and fumigation with rosemary (*Rosmarinus officinalis*) and Russian sage (*Perovskia atriplicifolia*) EOs was 3-6 times higher than when irradiation or EOs were used alone [104]. Packaging or inserts containing the plant EO in a polymer matrix for controlled release could be used for delivery. For phytosanitary uses, the vapor pressure of plant EOs is generally low, and so their ability to penetrate into commodities to kill internal-feeding insect pests (eg, fruit flies) may be limited. Therefore irradiation and plant EO combination treatments may be more practical for control of surface pests such as thrips, scale insects, mealybugs, and mites. Sensory analysis would be needed to identify any off flavors produced by exposure to the plant EO.

Combination with modified atmospheres

Long exposures to modified atmospheres involving low oxygen (O_2), high carbon dioxide (CO_2), high nitrogen (N_2) and other gases can be lethal to insects [105–107]. Exposure to these modified atmospheres can influence radiation tolerance in insects. For example, Buscarlet *et al.* [108] reported synergistic effects when *Tribolium confusum* was exposed to a nitrogen atmosphere before or after irradiation. However, low O_2 atmospheres such as those generated by MAP, may have the reverse effect. Irradiation damage in insects is partially caused by the creation of free radicals from oxygen and water which react with and damage nearby molecules, and insects are known to exhibit higher tolerance to radiation in low oxygen environments [109–111]. Low oxygen causes the insect to slow respiration thereby reducing O_2 in the hemolymph and reducing the creation of free radicals during irradiation. Low oxygen can also increase antioxidant capacity and decrease oxidative stress in insects [112]. Low oxygen concentrations generated with MAP may inadvertently reduce the efficacy of irradiation quarantine treatment against insects [**113, 114].

Several recent studies with tephritid fruit flies demonstrated the effect of simulated low oxygen conditions on radiation tolerance. A low oxygen atmosphere increased the estimated dose (from 30 to 35.7 Gy) to achieve 99% prevention of the full pupal stage in irradiated third instar apple maggots, *Rhagoletis pomonella* (Walsh), compared to ambient atmospheres [115]. A radiation dose of 200 Gy prevented adult emergence in 58,779 treated fifth instar oriental fruit moth, *Grapholita molesta* (Busck), in ambient atmosphere, but in an atmosphere flushed with nitrogen (presumably <1% O_2), 5.3% of adults emerged from 44,050 fifth instars [116]. Anoxia conditioning for 1 h applied to late stage Caribbean fruit fly, *Anastrepha suspensa* (Loew), pupae before irradiation at 70 Gy improved flight ability and male mating success [112]; anoxia also significantly improved adult emergence and percentage fliers when pupae received higher radiation doses of 200-400 Gy. Elevated CO_2 is not known to have a radio-protective effect [117, 118] unless it replaces oxygen as in the case of a 100% CO_2 environment [109–111].

Studies of insect radiotolerance under realistic conditions with fruit in MAP are rare. A sublethal radiation dose of 50 Gy applied to third instar melon flies infesting papaya inside MAP (1-4% O_2) increased survivorship to adult from 14 to 25% [**113]. Additional studies are needed with a variety of quarantine insect pests, especially surface pests and radiotolerant insects such as Lepidoptera.

Combination with pesticides

Insecticide exposure may modify insect response to irradiation. Although insecticide sprays or dips typically do not control internal feeding pests due to lack of penetration, insecticides may be useful for control of surface pests or as part of a system to control multiple quarantine pests on a commodity. For example, an irradiation and insecticide combination treatment may be practical if the internal pest is controlled at a low irradiation dose (eg, 150 Gy for fruit flies), but the surface pest requires a higher dose (eg, 250 Gy for thrips) and could be controlled with an insecticide dip. *Thrips palmi* in dendrobium orchids were effectively controlled by a combination of an insecticidal dip (100 ppm concentration of imidachoprid) and irradiation treatment at 350 Gy, and this combination treatment was used to successfully ship orchids from Thailand to Australia [119]. Azadiractin from the neem tree is an antifeedant and growth inhibitor with broad activity against insects; in the lepidopteran pest *Spodoptera litura*, larvae from irradiated (130 Gy) adult moths that were fed leaves treated with 1% azadiractin showed a greater reduction in growth, development and reproduction than larvae not exposed to azadiractin [120]. In both cases the irradiation dose was relatively high and therefore likely to reduce insect performance and survivorship by itself, and single treatment effects were not measured to determine if the combined treatment was synergistic. Disinfestation treatment of rice sacks for control of four rice pests with methyl bromide (150 ml/m^3) fumigation and irradiation at 75 Gy was equivalent to irradiation alone at 160 Gy [121]. Moustafa and Abdel Salam [122] reported that susceptibility of African (or Egyptian) cottonworm *Spodoptera littoralis* to chlorpyrifos was increased after exposure to sublethal doses at $\geq$100 Gy. *Spodoptera litura* larvae from irradiated parents were 4.2 times more susceptible to the insecticide thiodicarb than larvae from untreated parents [123]. The biopesticide *Bacillus thuringiensis* and irradiation showed additive and synergistic effects in studies with tropical warehouse moth *Cadra cautella* [124].

Further research into the use of low dose irradiation in combination with other technologies for insect disinfestation in fresh horticultural products could pay dividends. Particularly, irradiation in combination with cold may be a means to reduce the duration and therefore costs of current cold treatment protocols. Irradiation may also allow use of higher cold temperatures that do not cause chilling injury in cold-sensitive fruits. Additional research is needed to demonstrate the efficacy of irradiation plus cold combination treatments while assessing commodity quality.

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