Comprehensive REVIEWS in Food Science and Food Safety

Irradiation for Mold and Mycotoxin Control: A Review

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Abstract: The mycotoxin issue requires constant vigilance from economic, regulatory, and scientific agents to minimize its toxicological effects on human and animals. The implementation of good practices to avoid fungal growth and mycotoxin production on agricultural commodities is essential to achieve most restrictive safety standards; however, the contribution of novel technologies that may act on postharvesting and poststorage situations may be equally important. Several methodologies, more or less technologically advanced, may be used for this purpose. In this work, we review the role, contribution, and impact of irradiation technology to control the presence of fungi and mycotoxins in food and in feed. The effect of this technology on the viability of mold spores and on the elimination of mycotoxins is reviewed. A critical evaluation of the advantages and disadvantages of irradiation in this context is presented.

Keywords: feed, food, fungi, irradiation, mycotoxins

Introduction

The constant demand of consumers for safer, "healthier," and processed food drives the development of technologies in food processing to achieve their needs. Food safety is one of the major challenges for technology, although many preservation processes and regulations are already available to control the microbiological and chemical integrity of food. Food irradiation is one among many of available technologies that contribute to improve the safety of food.

Food irradiation is a physical method of food processing that involves exposing prepackaged or bulk foodstuffs to ionizing energy. This process is sometimes called "cold pasteurization" because the inactivation of microorganisms is achieved at low temperatures unlike the traditional heat pasteurization. Using irradiation, the microbiological safety of food can be improved and its shelf-life prolonged without substantially changing, in most cases, its nutritional, chemical, and physical properties. The elimination of pests on agricultural commodities can also be achieved, thus reducing food losses and the use of chemical fumigants and additives. Food irradiation up to an overall dose of 10 kGy has been considered a safe and effective technology since 1981 by several international food organizations (FAO/IAEA/WHO 1981). Later on, doses above 10 kGy were also considered safe for some niche products and markets (FAO/IAEA/WHO 1999). Nonetheless, food irradiation is not as widespread as other conventional technologies due to the high costs of irradiation units and, particularly, because of a negative perception of consumers relatively to its safety.

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The Food Irradiation Technology

Principles of radiation

Radiation is energy that originates from a source and that travels through most materials and through space. Light, heat, and sound are types of radiation (Satin 1996). Radiation is commonly classified according to wave frequency, for example, radio wave, microwave, infrared, visible light, ultraviolet radiation, X-rays, and γ-rays (Figure 1). The electromagnetic spectrum is also divided into 2 types of radiation: nonionizing radiation and ionizing radiation. The radiation discussed in this article is of the ionizing type. Ionizing radiation is produced by unstable atoms that have an excess of energy or mass or both and that reach stability by giving off these atoms or by emitting the excess energy or mass that these atoms possess.

The irradiation of food is a process where food is exposed to ionizing energy, such as γ photons emitted by ^{60}Co (or infrequently by ^{137}Cs) radioisotopes, X-rays generated by machines operated below a nominal energy of 5 MeV, and accelerated electrons generated by machines operated below a nominal energy of 10 MeV (Farkas and Mohácsi-Farkas 2011). Only these sources can be used for food irradiation because energies emitted by these sources are much too low to induce radioactivity in any exposed material.

Food irradiation using ^{60}Co is presently the preferred method because this method has a deep penetration capacity that enables the treatment of materials with less handling. Nonetheless, irradiators using ^{60}Co must be recalibrated on a monthly basis because of the continuing decay and concomitant loss of radioactive energy of this isotope (Prado 2005). Gamma-radiation can also be achieved by an isotope of cesium (^{137}Cs). In this case, this material is obtained by reprocessing and by extracting spent nuclear fuel from nuclear reactors. This fact has brought much criticism from nuclear opponents who claim that food irradiation was simply invented to

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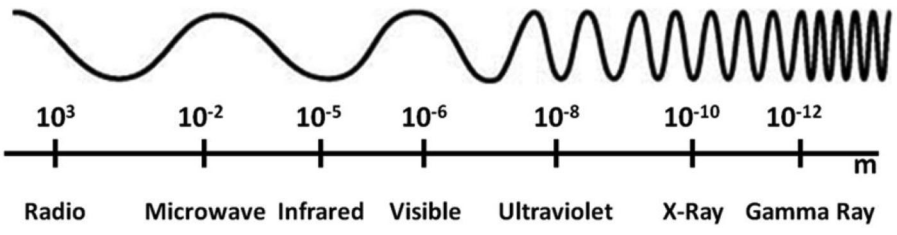

Figure 1–The electromagnetic spectrum (adapted from Satin 1996).

eliminate nuclear waste (Satin 1996). As a result, ^{137}Cs irradiators represent an extremely small proportion of today's irradiators. In contrast, the utilization of X-rays and electron beams involves the use of electrical machine sources of energy. An obvious advantage of such systems is that these systems can be switched on and off, similar to a light bulb, and are in no way related to the nuclear industry (Satin 1996). X-rays have low energetic efficiency, given that only 3% to 5% of the energy is converted to radiation, and electron beams have a limited penetration ability (Prado 2005). Typically, the penetration power of electron beams is only suitable for materials with a thickness of no more than 5 to 10 cm (Satin 1996). However, for certain uses, electron beams have proven to be extremely practical primarily for treating food surfaces, meat, and fruits.

Mechanism of action

Living cells are inactivated when exposed to factors that substantially change their cellular structure or physiological functions. Lethal structural damages include DNA strand breakage, cell membrane rupture, or mechanical damage to cell walls (Lado and Yousef 2002). During the irradiation of food, DNA is strongly damaged by radiation; therefore, primarily by this mechanism, microorganisms, insect gametes, and plant meristems are prevented from reproducing (Farkas 2006). DNA damage may result from a direct action of the ionizing radiation or from an indirect action of the oxidative radicals that originated from the radiolysis of cellular water (Farkas 2006).

The radiolysis of water takes approximately 10^{-6} s to occur. When water is irradiated by ionizing radiation, water molecules undergo a breakdown sequence that forms several radiolysis products that are extremely reactive with other chemical substances (Figure 2). The primary reactions that occur are the ionization and excitation of water molecules. Ionization causes the splitting of water molecules into positively charged water radicals (H_2O^+) and negative free solvated electrons (e^-); at the end of the process, due to various recombination and cross-combination reactions, the following reactive species are present: e^-_{aq}, $H^\bullet$, $HO^\bullet$, $HO_2^\bullet$, OH^-, H_3O^+, H_2, and H_2O_2 (Le Caër 2011).

Thus, these reactive species are free to react with any component present in the cell cytoplasm. The hydroxyl radicals remove hydrogen atoms from sugar and from the 4 bases of DNA strands (Lado and Yousef 2002). The other free radicals will also attack and break down organic molecules (Stepanik and others 2007). Potentially lethal DNA lesions are randomly scattered throughout the cell population during ionizing. The cells that are unable to repair their radiation-damaged DNA die (Lado and Yousef 2002). Differences in radiation sensitivities among microorganisms are related to differences in their chemical and physical structures and in their ability to recover from radiation injury (Farkas 2006). In general, the sensitivity of organisms to radiation increases with their complexity. Thus, the required radiation doses to achieve effective inactivation usually increase as follows: insects < parasites < molds and yeasts < vegetative (nonspore-forming) bacteria < spore-forming bacteria < viruses. Therefore, viruses are the most resistant to destruction by irradiation, and insects and parasites are the most sensitive. Moreover, spores (from bacteria and fungi) and cysts (from protozoa and parasites) are quite resistant to the effects of irradiation because spores and cysts contain very little DNA and are in highly stable resting states (Shea 2000). Therefore, the radiation energy required to control microorganisms on or in food varies according to the type of species to be eliminated, according to their population numbers and according to their developmental state. Other factors, such as the composition and moisture content of food, the fresh or frozen state of food, the temperature, and level of oxygen present during irradiation, may also influence the resistance of microorganisms to radiation, particularly in the case of vegetative cells (Farkas 2006).

Notably, during the irradiation of microorganisms, sublethally injured cells are often subject to mutations, and this occurrence can be dangerous. Mutations can result in greater, less, or similar levels of virulence or pathogenicity from parent organisms. The induction of radiation-resistant microbial populations occurred when cultures were experimentally exposed to repeated cycles of radiation (Shea 2000). However, mutations in microorganisms develop with any form of food processing (including ultraviolet light, heat, and drying). For this reason, it is extremely important to define safe irradiation doses to completely inactivate microorganisms (Shea 2000). One parameter most often used to compare the susceptibility of microorganisms to irradiation is the D_{10} value, namely, the dose required to inactivate 90% of a microbial population.

Nutritional and organoleptic adequacy

All forms of food processing affect nutritional and organoleptic properties, and irradiation is no exception. At doses below 1 kGy, the nutritional losses are considered insignificant. In contrast, the irradiation of food much above 10 kGy degrades nutrients similar to thermal processes, such as cooking, canning, pasteurizing, or blanching (Shea 2000).

Vitamin loss is the largest nutritional concern associated with food irradiation, particularly when synergism between irradiation and heat (cooking) occurs. Additionally, avitaminosis may arise when the irradiated commodity represents a large proportion of the dietary source of an essential vitamin (Shea 2000; Wood and Bruhn 2000). Water-soluble vitamins, such as the B vitamins and vitamin C, are the most affected because these vitamins are oxidized during irradiation (Shea 2000). Nonetheless, the loss of heat-sensitive vitamins with irradiation is considered no greater than that with conventional heat-processing and is often less. In

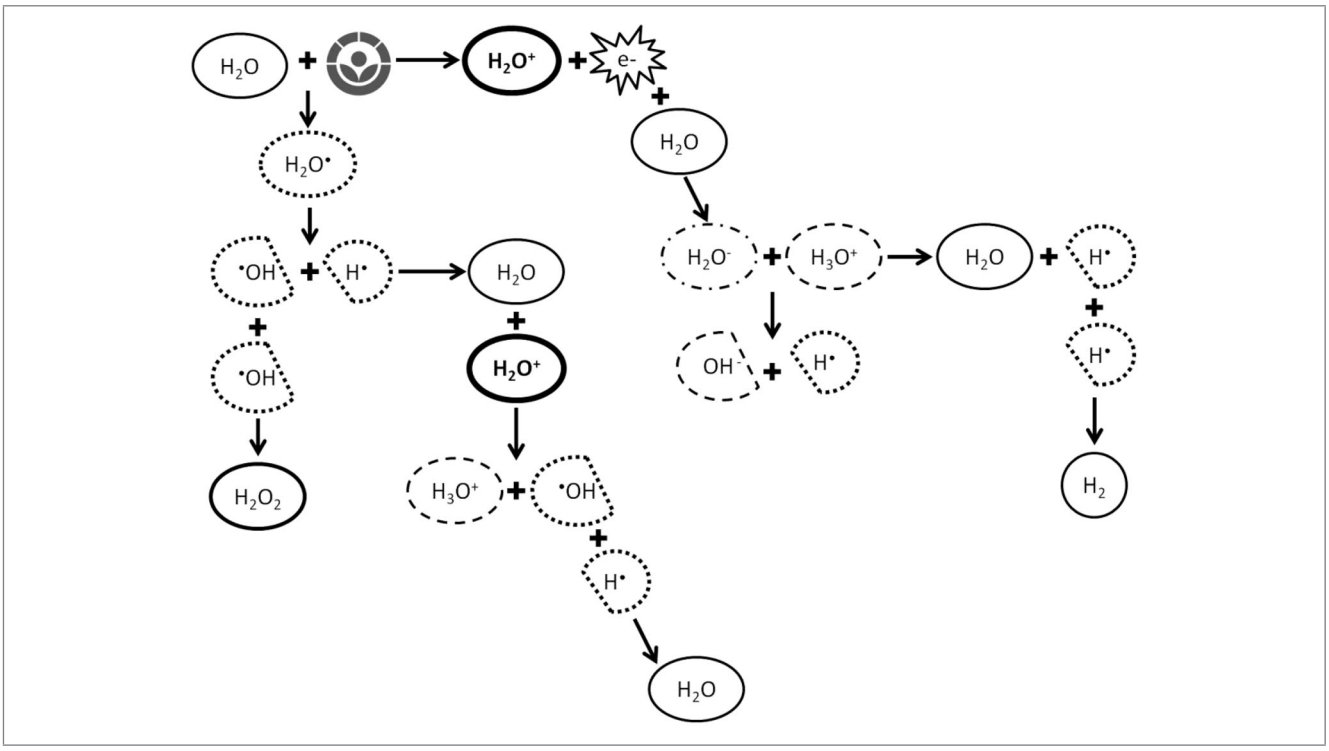

Figure 2–Reactions occurring during water radiolysis (adapted from de Campos and others 2004).

addition, research indicates that vitamin losses can be minimized by irradiating in oxygen-free packaging or at cryogenic temperatures ranging from −20 to −40 °C (EC 2003).

Carbohydrates are not significantly affected during irradiation at less than 10 kGy (Aziz and Mahrous 2004). In contrast to other preservation methods (such as pasteurization), protein denaturation is also not significant (Lado and Yousef 2002). A change in the bioavailability or quantity of minerals or trace elements has not been identified as a result of irradiation. Fats can be oxidized, leading to rancidity and to odor or color changes. In contrast, polyunsaturated fatty acids are not generally altered at low to medium irradiation doses (Shea 2000; Wood and Bruhn 2000). In addition to nutritional adequacy, organoleptic factors are also extremely important to the feasibility of food irradiation, particularly on fresh fruits and vegetables, which has been reviewed by Arvanitoyannis and others (2009).

Safety and legislative aspects

Food irradiation is not as widespread as other conventional technologies due to the high costs of irradiation units and, in particular, because of its unwarranted association with nuclear radiation, which gives consumers a negative perception of its safety. As a result, the health and safety of irradiated foods have been more exhaustively studied than any other processed food (Satin 1996). Since 1964, numerous international expert groups, which were jointly set up by the FAO, IAEA, WHO, and by the governments of different countries, collected and reviewed the scientific data produced over the years to consider the question of the wholesomeness of irradiated foods. The first international safety recommendation was presented in 1981, when a committee of experts considered that " . . . the irradiation of food up to an overall average dose of 10 kGy introduces no special nutritional or microbiological problems" (FAO/IAEA/WHO 1981). Then, the FAO/WHO Codex Alimentarius Commission developed the Codex General Standard for Irradiated Foods and the Code of Practice for Radiation Processing of Food (Codex 2003a,2003b). These documents became widely adopted internationally and, today, specific applications of food irradiation are approved by national legislations in over 55 countries worldwide (Farkas and Mohácsi-Farkas 2011).

Regarding the European Union, the implementation of food irradiation is far less developed when compared with other countries, such as the U.S.A., Brazil, or even China. In 2005, the European region represented only 4% of the world production of irradiated food (Kume and others 2009). This situation is primarily caused by the restrictive legislation in use. Directive 1999/2/EC, concerning the "approximation of the laws of the member states concerning food and food ingredients to be treated by ionizing radiation," and Directive 1999/3/EC, concerning "the establishment of a community list of foods and food ingredients treated with ionizing," are the main legislation pieces concerning food irradiation in the European community. In the first document, the European Parliament and the Council adopted a framework directive on the general and technical aspects of food and food ingredients treated with ionizing radiation (EU 1999a). In the second document, legislators established a list of foodstuffs authorized for irradiation treatment (EU 1999b). This list of foodstuffs is composed of 3 items: "dried aromatic herbs, spices and vegetable seasonings," and the permitted maximum overall average absorbed dose is 10 kGy. Since 2009, 7 EU Member States have issued authorizations to maintain their national regulation for food products, such as fruits and vegetables, including root vegetables; cereals, cereal flakes, and rice flour; spices and condiments; fish, shellfish; fresh meats, poultry, and frog legs; raw milk camembert; gum arabic, casein/caseinates, and egg white; and blood products (EU 2009).

Applications

From a practical point of view, 3 dose/application categories are typically considered when ionizing radiation is used to treat food: (i) a low dose of up to 1 kGy, which is used for sprout inhibition and to delay ripening and/or insect disinfestations; (ii) a medium dose from 1 to 10 kGy, which is used to reduce spoilage microorganisms, nonspore-forming pathogens, and/or to delay ripening; and (iii) a high dose from 10 to 50 kGy, which is used to eliminate microorganisms to the point of sterility for very specific products (Satin 1996).

Five groups are most often discussed when considering the type of application and of food irradiated. These 5 groups include the following: the disinfection of spices and dry vegetables, which represents 46% of all the irradiated products in the world; the sprout inhibition of garlic and potatoes, which represents 22%; disinfestations of grains and of fruits, which represent 20%; the disinfection of meat and seafood, which represents 8%; and the treatment of other food items, such as health foods, mushrooms, or honey, which represents 4% (Kume and others 2009). The total quantity of food irradiated in the world in 2005 was approximately 405000 metric tons.

Irradiation is also used to eliminate or to reduce the presence of pathogenic microorganisms, such as *Aeromonas hydrophila*, *Arcobacter butzleri*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, yeasts, molds, and others, in several food matrixes (Farkas 1998, Stefanova and others 2010).

Advantages and disadvantages

The primary advantage of food irradiation is most likely the nonresidual feature of the process. In contrast to chemical methods, which leave residual components that may have or are suspected to have a negative impact on human health, irradiation is free of chemical residues. An example is the quarantine treatments required to mitigate pests from fruits, vegetables, and other plant-derived materials (Ferrier 2010). Irradiation is an excellent substitute for the conventional fumigation in use. Additional advantages of irradiation technology include: the possibility (i) of irradiating packed food at its fresh and frozen state; (ii) of controlling the hygienic quality of food by eliminating pathogenic and nonpathogenic microorganisms, insects, and parasites; (iii) of extending the shelf life of foods, thus increasing its supply, and (iv) of preserving the fresh-like quality of agricultural commodities because irradiation technology is a cold-processing method (Stefanova and others 2010). Furthermore, irradiation may be considered environment friendly because this method does not consume water and has lower electrical energy demands than other food preservative methods, with exception of electron beam and X-ray radiation, which are energy costly.

Concerning the disadvantages of this method, first, irradiation cannot be applied to all types of foods. Some fruits, such as pears and plums, vegetables, milk, and dairy products are untreatable by irradiation because these products lose firmness and some important sensory and other quality properties (Stefanova and others 2010). In other cases, irradiation can originate minor changes in the nutritional and organoleptic characteristics of treated foods as mentioned above. Most relevant for foods is the reduction of water- and fat-soluble vitamin contents; the production of off-flavors, lipid oxidation, and changes in color; the creation of oxidation compounds, such as aldehydes, ketones, and alcohols; and the formation of radiolytic products, particularly 2-alkylcyclobutanones, which are suspected to be toxic (Stefanova and others 2010). Nonetheless, most of these changes are also induced by traditional food preservative methods, such as cooking, canning, pickling, freezing, and drying. In addition, radiolytic products, such as 2-alkylcyclobutanones, have also been found on nonirradiated food products, contradicting previous beliefs (Variyar and others 2008).

The fact that food irradiation can cause the mutation of pathogenic microorganisms and could create new resistant strains can also be perceived as a disadvantage. However, the risks posed by this occurrence are minimal and comparable with those posed by other food processing methods (Farkas 1989). A further disadvantage of food irradiation is that this method can be globally more expensive than other preservative methods due to the upfront costs of food irradiation facilities. The poor acceptance of these products by consumers because of unwarranted fears that associate food irradiation with nuclear technology is also an opposing threat. Nevertheless, consumers´ trust of this technology can be changed through education, provided that stakeholders disseminate conscious and scientifically rigorous information concerning the subject.

Irradiation to Control Mold Growth and Mycotoxins

The mold and mycotoxin issue

Filamentous fungi are a large group of eukaryotic microorganisms that are associated with an enormous diversity of habitats. Many of them are saprophytes, which are responsible for the deterioration of agricultural products and food. Saprophytes may be responsible for the decay of commodities at a preharvest level; however, saprophytes may also be responsible for the deterioration of fresh and processed foods, causing their rejection due to the visible development of molds on these foods. In addition, some specific fungal species growing on agricultural commodities may produce mycotoxins. Mycotoxins are a hidden aspect of mold contamination because they remain on or in products well beyond the life cycle of the fungi. Moreover, mycotoxins are extremely stable and moderately heat-resistant compounds that remain almost intact after food processing (Bullerman and Bianchini 2007).

Mycotoxins are fungal secondary metabolites found in many plant foodstuffs, particularly in cereals, fruits, nuts, kernels, seeds, and animal fodder, and are toxic for humans and animals when ingested or inhaled with dust. The most relevant mycotoxins for food safety are aflatoxins (AFs), ochratoxin A (OTA), patulin, fumonisins, zearalenone (ZEN), and trichothecenes. These mycotoxins are produced by some species from the genera *Aspergillus*, *Penicillium*, and *Fusarium* and have multiple and combined toxic characteristics. These mycotoxins may be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, immunosuppressive, and/or estrogenic (Paterson and Lima 2010).

Typically, AFs are the most well-recognized and studied mycotoxin. AFs are highly carcinogenic and hepatotoxic (Williams and others 2004) and are primarily found in peanuts, maize, nuts, spices, and in milk (where this mycotoxin occurs from the B form in feed to the AF M form). OTA is primarily known for its nephrotoxicity; however, OTA is also carcinogenic to experimental animals (Pfohl-Leszkowicz and Manderville 2007). OTA is mostly found in cereals and in cereal-based products; however, OTA also occurs in coffee beans, nuts, spices, raisins, and in red wine (Jørgensen 2005). Patulin is primarily associated with fresh fruits and vegetables. Apples, apple juices, and purees are the main dietary sources of this mycotoxin. Patulin is neurotoxic, immunosuppressive, genotoxic, and teratogenic (Moake and others 2005). Fumonisins, which primarily occur in maize and in maize-based

food products, appear to be related to an increased incidence of esophageal cancer and liver cancer in humans and are experimentally associated with leukoencephalomalacia in horses and with pulmonary edema syndrome in pigs (Voss and others 2007). ZEN is estrogenic and interferes with the reproductive system of animals, even if ZEN has a relatively low acute toxicity (Zinedine and others 2007). Similar to fumonisins, ZEN is primarily associated with maize and with maize-based food products. Trichothecenes are a large group of structurally related compounds. The most relevant trichothecenes for food safety include T-2 toxin, HT-2 toxin, deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (ADON), and nivalenol (NIV) (Foroud and Eudes 2009). These compounds are primarily found in cereal grains and are extremely cytotoxic to mammalian cells, initiating a wide range of toxic effects, such as digestive disorders, followed by diarrhea and by vomiting (Foroud and Eudes 2009).

Therefore, if the presence of mycotoxins in food and feed is not properly controlled, mycotoxins may pose important risks to public health. At low levels, mycotoxins may cause the suppression of immune functions and decrease resistance to infections in individuals. In acute situations, mycotoxins may cause the development of tumors and of chronic diseases in vital organs, or high morbidity and premature death among humans and animals (Peraica and others 1999). Additionally, mycotoxins are also responsible for major economic losses at all levels of the food-production chain. These losses are primarily associated with the rejection and destruction of contaminated materials and with expenses incurred toward the implementation of good postharvested storage conditions, analyses, and treatments to guarantee low levels of mycotoxins.

To avoid the introduction of most contaminated products into the food chain, the presence of mycotoxins in certain agricultural commodities and finished foods and feeds are regulated by statutory levels in many countries of the world (van Egmond and others 2007). Additionally, a great diversity of preventive and corrective measures can be applied to control the problem. The preventive measures may include HACCP integrated systems, which involve strategies for prevention at pre- and postharvested levels, good manufacturing practices, and quality control. The corrective measures include several physical, chemical, and biological decontamination techniques that promote the elimination of the contaminated fraction or that counteract the toxic effects of mycotoxins (Stoev 2013). Next, we will review how food irradiation can contribute to this purpose.

Irradiation to control mold growth

As already observed, molds are one of the main causes of postharvest decay problems. The presence of molds in food may result in not only a reduction in quality and in quantity but also contamination with mycotoxins, causing important health problems. Irradiation can be used for the direct purpose of eliminating or of reducing the presence of molds and mold spores in foods and in feeds, improving their shelf life and safety. Nonetheless, the application of this technology for other purposes can indirectly aid in the control of contamination with molds and, subsequently, with mycotoxins. For example, it is well known that grains damaged by insects are more susceptible to mold development and to mycotoxin accumulation because insects carry fungal spores and compromise the integrity of grains and plant tissues, facilitating the penetration and access to nutrients of fungal hyphae and, by consequence, fungal development (Jouany 2007). Thus, the elimination of insect pests from agricultural commodities through irradiation can indirectly have a positive preventing effect on the reduction of fungal contamination and mycotoxin levels in treated commodities. However, importantly, the irradiation of disinfestations of grain must be combined with good grain handling practices so that mycotoxin production can also be prevented during storage.

Concerning the direct action of irradiation on molds associated with foods and with feed, many reports are available in the literature that evaluate its effect, specifically on spices and on dried vegetables, which are the most irradiated food items worldwide. A study evaluating the effect of gamma-irradiation on the fungal load in red chillies was conducted by Iqbal and others (2013) and concluded that irradiation doses of 6 kGy were sufficient to reduce the fungal load by 5 logs. Another study conducted on hot peppers observed reductions by 1 and 2 logs of the fungal load with doses of 2 and 4 kGy, respectively (Iqbal and others 2012). With a dose of 6 kGy, no molds were detected. Similarly, Legnani and others (2001) studied the effect of gamma-irradiation on the microbiological qualities of black pepper, red chili, oregano, rosemary, and sage. In this study, radiation doses of 5 kGy were suitable to significantly reduce the load of molds (between 65% and 80%); however, their complete elimination was only achieved with 10 kGy. In this case, *Aspergillus niger*, *Cladosporium* spp., *Penicillium* spp., and *Rhizopus* spp. were the most resistant to irradiation doses of 5 kGy. Similar results were obtained by Farag and others (1995) who studied the effect of irradiation on marjoram, ginger, and hot pepper. These authors reported the complete elimination of molds and, specifically, of *Aspergillus flavus* (a producer of AFs) with a radiation dose of 10 kGy. Coriander, cumin, turmeric, and chili were also submitted to irradiation experiments by Alam and others (1992) who obtained D_{10} values for molds that ranged from 0.71 to 2.14 kGy, depending on the spice studied. In this case, an irradiation dose of 5 kGy was considered sufficient to control fungal contamination because no molds were detected in samples after 3 and 6 mo of storage.

As can be observed from these studies, a substantial reduction of the fungal load in spices and in seasonings is only achievable with irradiation levels above 5 kGy. In this case, the high levels of irradiation does not seem to affect the quality of products because no losses of flavor compounds, changes in volatile oil compositions, and weakening of antioxidant properties at irradiation levels of 10 kGy or even 30 kGy were found by several researchers and reviewed by Alam and Abrahem (2010). Thus, the irradiation of spices is widely used as an excellent substitute to fumigation with gases, such as ethylene, propylene oxide, or methyl bromide, which leave chemical residues (for example, ethylene chlorohydrins and ethylene bromohydrin) that are suspected to be harmful. The dried nature of these products may be the factor that favors their greater resistance to the ionizing energy.

Concerning the irradiation of grains, pulses, and seeds, the inactivation of molds in rough rice and in wheat through gamma-irradiation was reported by Wang and Yu (2010). In wheat, an irradiation dose of 3 kGy was sufficient to reduce the presence of *Alternaria*, *Aspergillus*, and *Fusarium* 10-fold. After irradiation, *Penicillium* and *Rhizopus* species were not detected. In rice, the irradiation dose required to obtain the same effect was 5 kGy. After irradiation, the detection of molds had not significantly changed during storage periods of 6 and 12 mo. Maity and others (2008; 2009) also evaluated the effect of gamma-irradiation on the fungal diversity of rice seeds during storage periods up to 12 mo. The growth of isolated fungi was completely inhibited during this period with a 3-kGy dose, and no change in the germination potential was noted with doses ranging from 2 to 4 kGy. Aziz and

others (2006b) studied the effect of gamma-irradiation on wheat, barley, maize, and sorghum and reported that fungi were completely inhibited by a dose of 5 kGy. In this study, bacteria were more resistant to radiation than molds, leading the authors to conclude that a radiation dose of 10 kGy was required to improve the total hygiene of grains. Furthermore, no significant alteration of their nutritional constituents was observed. In later study, Aziz and others (2007) assessed the control of *Fusarium* species on wheat, barley, and maize seeds. *Fusarium* spp. were completely inhibited at 4.0 kGy on barley and at 6.0 kGy on wheat and on maize. A 6-kGy dose could also completely inhibit the fungal population in several types of grains; however, 4.0 kGy only reduced the fungal load by 4 logs (Aziz and others 2006a). Aziz and Moussa (2004) also verified that gamma-irradiation at a dose of 5 kGy inactivated the growth of molds and subsequent mycotoxin formation in maize, chick-peas, and groundnut seeds. In contrast, in ground and whole maize, D´Ovidio and others (2007) found that radiation doses of 30 and 100 kGy, respectively, were required for the complete inactivation of *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.; and a major reduction in the mold load was also observed with lower doses, at 10 and 30 kGy, respectively. This work did not agree with previous studies that also evaluated the effect of irradiation on maize. For example, Webb and others (1959) found that radiation doses between 2.5 and 10 kGy were sufficient to prevent the growth of molds in maize during storage and that the required dose increased with maize moisture content. In this study, molds that required higher moisture levels to grow were also more resistant to gamma-radiation. Similarly, Ferreira-Castro and others (2007) reported that *Fusarium verticillioides* survival percentages on maize irradiated with 2, 5, and 10 kGy were 36%, 6%, and 0%, respectively; thus, a 5-kGy dose could substantially contribute to the avoidance of maize contamination with this species. A radiation dose of 1.5 kGy was also found to reduce the maize fungal population by 90% and 99%, depending on the maize moisture content, which was 35% and 39%, respectively, in this case (Poisson and others 1971).

The effect of irradiation on sesame seeds was studied by Akueche and others (2012). An irradiation dose of 3 kGy inactivated 75% of molds present, and doses above 6 kGy completely eliminated any fungal development. Cowpea bean grains were studied by Lima and others (2011). Irradiation has been shown to be an effective method to preserve this variety of bean for 6 mo. The results indicated that *A. niger* was eliminated with 1.0 kGy; *Aspergillus ochraceus* with 2.5 kGy, *A. flavus* with 5 kGy; and fungi from the genera *Rhizopus*, *Penicillium*, and *Fusarium* with a 10-kGy radiation dose. Lotus seed irradiation was studied by Bhat and others (2010). Irradiation with a 7.5-kGy dose substantially reduced fungal contamination, and a 10-kGy dose completely eliminated fungi. In this case, contaminant yeasts were the most resistant to irradiation because some survived 10-kGy treatments. Kottapalli and others (2003) used electron-beam irradiation to reduce malting barley infection with *Fusarium*, observing that doses higher than 4 kGy effectively reduced the fungal infection without affecting its germination. Zeinab and others (2001) reported that a 6-kGy dose could completely inhibit the fungal population of *Nigella sativa* seeds (black cumin).

Concerning feed, gamma-irradiation was used, for example, to extend the shelf life of hydrated feed for fish farming without using preservatives (Kim and others 2012). A 5-kGy dose was sufficient to eliminate molds. Ribeiro and others (2009) studied the effect of gamma-radiation on the mycoflora of poultry feed. The total elimination of mold viability was observed at 8 kGy; however, *Aspergillus parasiticus* and *A. flavus* were the most resistant to irradiation, which may pose some safety concerns because these species are AF producers. Refai and others (1996) studied the elimination of *A. ochraceus* from poultry feed concentrate using gamma-radiation and concluded that a dose of 4 kGy could completely inhibit this species and the production of OTA. Similarly, El-Far and others (1992) studied the inhibition of *A. flavus* in the poultry diet, reporting that no fungal growth and AF production was observed at a 6-kGy dose. In contrast, Paster and others (1991) reported that irradiation doses of 7 to 10 kGy delayed fungal development, particularly in feed grains with low moisture content, but did not completely prevent the moldiness of this product.

Another application of irradiation technology focuses on the treatment of fresh fruits and vegetables to increase shelf life. Aziz and Moussa (2002) studied the effect of gamma-radiation on 10 different type of fruits refrigerated at <10 ºC for 28 d. The initial viable mold population ranged from 4.8×10^4 to 6.8×10^5 CFU/g. When fruits were submitted to 1.5- and 3.5-kGy doses, the initial mold population was reduced on average by 2 and 3 logs, respectively. Nonetheless, no studies were conducted to evaluate the quality and physicochemical parameters of the irradiated fruits. More recently, Mostafavi and others (2012) studied the influence of low--irradiation doses on apple preservation. The results demonstrated that the germination of spores from *Penicillium expansum* was completely inhibited with a 0.6-kGy dose and that doses between 0.3 and 0.6 kGy, which were combined with storage at 1 ºC, could avoid the development of rot for 9 mo without significantly changing the phenolics content, antioxidant activity, firmness, weight loss, and total soluble solids of the treated apples. Ben-Arie and Barkai-Golan (1969) showed that the inactivation of *P. expansum* could also be achieved in pears using a treatment with hot water (47 ºC for 7 min), followed by gamma-irradiation at 0.5 kGy. The same treatment did not prevent the rotting of fruits inoculated with *Botrytis cinerea* and with *Alternaria tenuis*; however, a delay in disease development was observed. Kim and others (2010) studied the effect of gamma-radiation on peach. A dose of 1 kGy inactivated *B. cinerea*, *P. expansum*, *Rhizopus stolonifer* var. *stolonifer*, and *Monilinia fructicola* in peach pulp, and the calculated D_{10} values for each species were 0.15, 0.23, 0.16, and 0.16 kGy, respectively. El-Samahy and others (2000) studied the microbiological and chemical properties of irradiated mango. An increased reduction in the fungal population on mango fruits was observed with irradiation doses increasing from 0.5 to 1.5 kGy. The ideal treatment reported by the authors involved dipping mangos in hot water (55 ºC for 5 min) and irradiating with 1 kGy. With these conditions, the ripening of mangos could be delayed for 50 d at 12 ºC without significantly changing their nutritive and sensory properties. A similar treatment for tomatoes was found to reduce *B. cinerea*, *R. stolonifer*, and *Alternaria alternata* decay (Barkai-Golan and others 1993). In this case, a hot water dip at 50 ºC for 2 min and an irradiation of 1 kGy were required. Nonetheless, the treatment caused a more rapid softening of fruits. The shelf life of strawberries could also be extended for 2 to 3 d with irradiation doses of 2 to 3 kGy when preserved at 23 ºC and for 4 to 8 d when preserved at 8 ºC (Shibaba and others 1967). In this work, lethal doses for 2 different strains of *B. cinerea* were found at 9.7 and 5.4 kGy, showing that resistance to irradiation may vary within the same species. Under the same experimental conditions, lethal doses for *Penicillium* sp. and *Aspergillus* sp. were 2 to 2.5 and 4 kGy, respectively. Ladaniya and others (2003) studied the influence of gamma-radiation on citrus fruits and concluded that radiation treatments could not reduce the decay of these products. Positive

effects were only observed in mandarins. In this case, *Penicillium* rot could be delayed with a radiation dose of 1.5 kGy, whereas no significant changes in fruit firmness and in juice content were observed. However, total soluble solids increased, whereas acidity and vitamin C content decreased. In oranges and in limes, radiation treatments considerably changed the texture and appearance of fruits. In fact, in the literature, the irradiation of citrus fruit resulted in injuries that led to the development of black buttons on the skin and, later, to the development of rot (Maxie and others 1964; O'Mahony and others 1985). Macfarlane and Roberts (1968) also concluded that irradiation is satisfactory for the disinfection of orange fly because the required doses are extremely low; however, this method is not feasible for mold control because of injury provoked by the high doses required. According to these authors, an irradiation dose of 0.3 kGy should not be exceeded for citrus fruit treatment. A review of the impact of ionizing radiation on fruits and vegetables can be consulted for more information concerning the subject (Arvanitoyannis and others 2009).

Concerning dried fruits, the irradiation of peanuts was evaluated by de Camargo and others (2012) who concluded that an irradiation level of 5.2 kGy was suitable to prevent the growth of aflatoxigenic fungi without significantly affecting their polyunsaturated fatty acid and polyphenol contents. This observation agreed with previous data from Chiou and others (1990), which proved that radiation levels of 2.5 and 5.0 kGy were effective in retarding the growth of *A. parasiticus* and in reducing the native mold population of peanuts, respectively. Additionally, Hilmy and others (1995) reported that 3.0 and 5.0 kGy could completely inhibit *A. flavus* growth on peanut and nutmeg meal, respectively. In contrast, Prado and others (2006, 2003) observed only a reduction in fungal infections on peanuts irradiated with 5 kGy and its total elimination only with 10 kGy. The effect of gamma-irradiation on the quality of walnuts was also studied (Wilson-Kakashita and others 1995). The mold count in walnuts was significantly reduced with irradiation doses above 5 kGy, which were shown to be more effective then propylene oxide treatments. Walnut lipid contents did not changed with gamma-radiation treatments; however, a small decrease in iodine contents and an increase in peroxide values were observed. Similarly, Emam and others (1994) compared the irradiation of semidry date fruits with methyl bromide treatments and concluded that irradiation at 3 kGy was more effective at inhibiting the growth of fungi, despite causing a significant loss in weights of dates.

Concerning the direct effect of ionizing radiation on fungal species, Ribeiro and others (2011) studied the effect of gamma-radiation (at 2 kGy) on *A. flavus* and on *A. ochraceus*. Irradiated strains showed different color and slight differences on the sizes of stipes, metulae, and conidia compared with the same nonirradiated strains. The authors also observed that irradiated strains produced 2 times more mycotoxins than control strains. A similar effect was observed by other researchers. Irradiated strains of *A. flavus*, *A. parasiticus*, *A. niger*, and *A. ochraceus* also produced more AFB_1 or OTA and then nonirradiated strains (Schindler and others 1980; Ribeiro and others 2009). However, this finding is not a consensus observation because other researchers reported the opposite response; for example, irradiated spores of *A. parasiticus* did not produce more AFB_1 than nonirradiated spores on rice (Sharma and others 1990). In addition, it was also observed that the increase of mycotoxin production by irradiated spores was due to the reduction of spores' number, because serially diluted spores by 4 to 5 log produced also more mycotoxin (Sharma and others 1980). Even so, it is recommended that appropriate storage practices are implemented after the irradiation process to avoid the proliferation of toxigenic fungi and the associated production of mycotoxins.

Maity and others (2011) evaluated the effects of gamma-radiation on fungi isolated from rice. The responses of individual cultures of *A. alternata*, *A. flavus*, *Trichoderma viride*, and *Curvularia geniculata* submitted to irradiation doses up to 4.2 kGy were evaluated. The inactivation of fungal viability was achieved at 2 and 3 kGy for *T. viride* and for *A. flavus*, respectively, and at 2.5 kGy for *A. alternata* and for *C. geniculata*. Additionally, the following major changes in fungal morphology were observed: a reduction in colony radial growth, a reduction in the germination tube length and diameter, and, in some cases, multigerm tube formation. Similarly, Saleh and others (1988) reported the gamma-radiation doses required to inactivate some fungal species. In this case, dematiaceous fungi with melanized mycelia and conidia, such as *Alternaria*, *Curvularia*, and *Cladosporium*, were more resistant to gamma-radiation, and the reported inactivation doses were 11.5 to 13.9, 17 to 20, and 6.0 to 6.5 kGy, respectively, for each of these fungi. In contrast, *A. niger*, *Aspergillus fumigatus*, *A. parasiticus*, *Fusarium solani*, and a *Penicillium* sp. were inactivated by doses of 1.7 to 2.5 kGy, and *A. flavus* was inactivated by doses of 2.5 to 3.0 kGy. The effect of gamma-radiation on *A. flavus* and on *A. parasiticus* was also studied by Kume and others (1989). In humid conditions, these authors obtained D_{10} values of approximately 0.27 to 0.29 kGy for both species, whereas in dry conditions, the doses required to reduce the load by 1 log were almost double (0.5 to 0.6 kGy). This study showed that dry spores were more resistant to gamma-radiation. The same observation was also reported by other authors (Poisson and others 1971; Chang and Lee 1980; LebaiJuri and others 1995). Gumus and others (2008) studied 2 heat-resistant molds, *A. fumigatus* and *Paecilomyces variotii*, which were isolated from margarine. The average D_{10} value obtained for *A. fumigatus* was 1.08 kGy, whereas this value was 0.59 kGy for *P. variotii*. The complete inactivation of *P. variotii* was achieved with 5 kGy, whereas a 7-kGy dose was required for *A. fumigatus*. The radiation sensitivities of *A. flavus*, *A. niger*, a *Penicillium* sp., *B. cinerea*, and *R. stolonifer* were also evaluated by Chang and Lee (1980). *Aspergillus flavus*, *A. niger*, and *Penicillium* sp. presented a similar D_{10} value (0.3 to 0.35 kGy), whereas *B. cinerea* and *R. stolonifer* showed D_{10} values of 0.55 and 1.0 kGy, respectively. Malla and others (1967) reported that *Penicillium viridicatum* strains were more sensitive to gamma-radiation than strains of *A. flavus*. Their total inhibition was obtained with a dose of 2 kGy. The authors also found that spores of strains with 6-mo-old cultures were more susceptible to irradiation than 3-wk-old cultures. Aziz and Moussa (2004) reported D_{10} values in saline solutions for *A. alutaceus*, *A. flavus*, and for *F. verticillioides* of 0.36, 0.52, and 0.87 kGy, respectively. Similarly, Geweely and Nawar (2006) evaluated the effect of gamma-radiation on *Alternaria tenuissima*, *B. cinerea*, *P. expansum*, and *Stemphylium botryosum*, which are pathogenic to pears. *B. cinerea* and *P. expansum* were more radiosensitive, with complete inactivation by a 1.0-kGy dose, whereas *A. tenuissima and S. botryosum* were only inactivated by a dose of 3.0 kGy. LebaiJuri and others (1995) reported D_{10} values for many species that are pathogenic to plants. For *Fusarium* species, radiation doses required to reduce the load by 1 log were between 0.31 to 0.71 kGy. The most radioresistant species was *F. moniliforme*, whereas *F. oxysporum* showed the greatest potential for recovery after irradiation. These species are known to produce several mycotoxins, and their resistance to irradiation may raise some concerns

for fungal development after treatments if the radiation doses used are not sufficient to completely eliminate these species.

As we have observed, the radiosensitivity of a specific fungal species may be substantially different, depending on the works consulted. These differences may result from innumerous factors whose influence has not been as extensively studied as, for instance, the simple effect of radiation on the fungal load in specific food matrixes. Such factors may include the form of fungal contamination (mycelium or spores), the moisture contents of spores or commodities, the age of spores, the nature of the substrate on which radiation treatments are performed, the existence of periods of refrigeration or of heating before or after treatments, and the combinations of radiation with other technologies. These factors are summarized in Figure 3. In general, dried spores are considered more resistant to radiation, as we have already discussed; however, commodities with high-moisture contents may favor fungal recovery after irradiation if inactivation is not complete. The effectiveness of irradiation also depends on the age of the spores. Spores more than 1 mo old and less than 5 mo old were substantially more resistant to gamma-radiation (Poisson and others 1971). These authors also observed that radiosensitivity increased with moderate heating (40 to 50 ºC) before irradiation and with fungicide treatments (Poisson and others 1971). When experiments were conducted in inert supports rather than on nutritive media, the radiosensitivity of spores was also higher. Münzner (1969) reported additional observations, namely, that the recovery of irradiated spores was favored on optimal nutritive media by optimal incubation temperatures and that actively growing cultures of the molds were more sensitive to radiation than older cultures. A substantial difference in radiosensitivity may also be observed, depending on the strains tested. For example, for 2 different *B. cinerea* strains, gamma-radiation lethal doses were extremely different (9.7 and 5.4 kGy) (Shibaba and others 1967). Thus, a comparison of the susceptibility of fungal species to irradiation should be performed with care because numerous factors may change their susceptibility, particularly when the irradiation of natural substrates is involved.

Irradiation to control mycotoxins

Because mycotoxins are highly toxic, it is imperative that their levels in food and in feed are reduced as low as technologically feasible. Ionizing radiation is one among many technologies that can contribute to this purpose. As we have observed, first, its action on mold viability contributes to the avoidance of fungal development and, consequently, to the production of mycotoxins in commodities. Second, because ionizing radiation can have a direct action on mycotoxins under specific conditions, contributing to their elimination, this subject has been widely investigated, particularly concerning AFB_1. Nonetheless, the available literature is not always in agreement because some reports claim substantial reductions in some mycotoxins through the action of irradiation, whereas others claim that irradiation is not effective at all. Next, we will review the main achievements reported for this subject.

One of the first reports studied the effect of gamma-radiation on dried AFB_1 and on AFG_1 spotted on TLC silica plates and solubilized in phosphate solutions (Frank 1970). The authors observed that dried AFLs were extremely radioresistant, whereas in solution, AFLs were sensitive to irradiation doses of 1 and 2.5 kGy, with degradation of approximately 90%. Later, Van Dyck and others (1982) studied the radiosensitivity of AFB_1 in water solutions and showed that an identical irradiation dose could eliminate only 34% of the mycotoxin. Furthermore, the authors observed that increasing doses of radiation could destroy increasing amounts of AFB_1 until its total destruction at 20 kGy. Using a test with *Salmonella typhimurium* TA 98, these authors also demonstrated that AFB_1 mutagenicity decreased with increasing doses of gamma-radiation. Nonetheless, when the concentration of AFB_1 was increased 50 times, the effect of gamma rays was substantially lower, indicating that the mycotoxin concentration is a determinant factor to achieve satisfactory elimination percentages. Similar observations were later reported by Mutluer and Erkoç (1987) who studied the effect of gamma-radiation on AFB_1, AFB_2, AFG_1, and AFG_2 in solutions of water/DMSO. AFB_1 was the most radiosensitive, and AFB_2 was the most resistant. Irradiation doses of 5, 10, and 20 kGy were studied. AFB_1 and AFG_1 were almost completely eliminated at 5 kGy, retaining 5% and 10% in solution, respectively. In contrast, 90% of AFB_2 and 77% of AFG_2 were resistant to the same radiation dose. With 10 and 20 kGy, AFB_1 could be completely eliminated; however, AFG_1 was only completely eliminated with the 20-kGy dose. Patel and others (1989) also used this approach and investigated the synergetic effects between hydrogen peroxide and gamma-radiation on the elimination of AFB_1 in aqueous solutions. In the presence of 5% H_2O_2, these authors observed that a 1-kGy dose could eliminate 50 μg of AFB_1 and that a 4-kGy dose could eliminate 100 μg. The mycotoxin mutagenicity was also completely lost with 4 kGy in the presence of 5% H_2O_2 using an Ames microsomal test with *S. typhimurium* TA100. Additionally, these authors confirmed that artificially contaminated groundnuts could be detoxified using this strategy because these authors observed reductions of AFB_1 from 14 to 3 μg/g and from 6.3 to 1.7 μg/g in treated samples. Despite the observation that AFLs were degraded by gamma-radiation, no degradation products were identified, although their presence in samples was observed using TLC in some cases. Recently, Wang and others (2011) approached this subject using gamma-irradiated solutions of AFB_1 in methanol/water. Twenty different radiolytic products were obtained; however, only 7 products were tentatively identified. Using the quantitative structure–activity relation, 6 of the 7 radiolytic products were considered less toxic than AFB_1 because these products lost the double bond in the terminal furan ring, which is the determinant for AF toxicity. Using chicken embryos, the lethality of AFs was observed to decrease with increasing gamma-irradiation doses (Ogbadu and Bassir 1979).

As we have observed, AFB_1 in solution can be effectively degraded and detoxified using gamma-radiation. Most likely, this degradation is mediated by the oxidative radicals that originated from water radiolysis because dried AFB_1 is more resistant to radiation than AFB_1 in solution (Frank 1970). This possibility can be a limiting factor when radiation is applied to food and to feed products with the purpose of eliminating mycotoxins. Thus, studies in real matrixes are required to evaluate the true effect of irradiation on mycotoxins.

Iqbal and others (2013) evaluated the effect of gamma-radiation on the reduction of AFLs in chillies and observed that levels of AFLs decreased with increasing irradiation doses (2, 4, and 6 kGy). The highest reductions obtained ranged from 81% to 91% and were achieved with a 6 kGy dose. In contrast, Akueche and others (2012) did not observe any consistent reduction of AFLs and of OTA on irradiated sesame seed grains at doses ranging from 3 to 12 kGy; however, these authors obtained the lowest mycotoxin level on grains irradiated with a 15-kGy dose. Jalili and others (2012) also studied the effect of gamma-radiation on AFL and OTA contents on pepper. The tested doses ranged from 5 to 30 kGy. The greatest reductions in mycotoxin levels (35% to

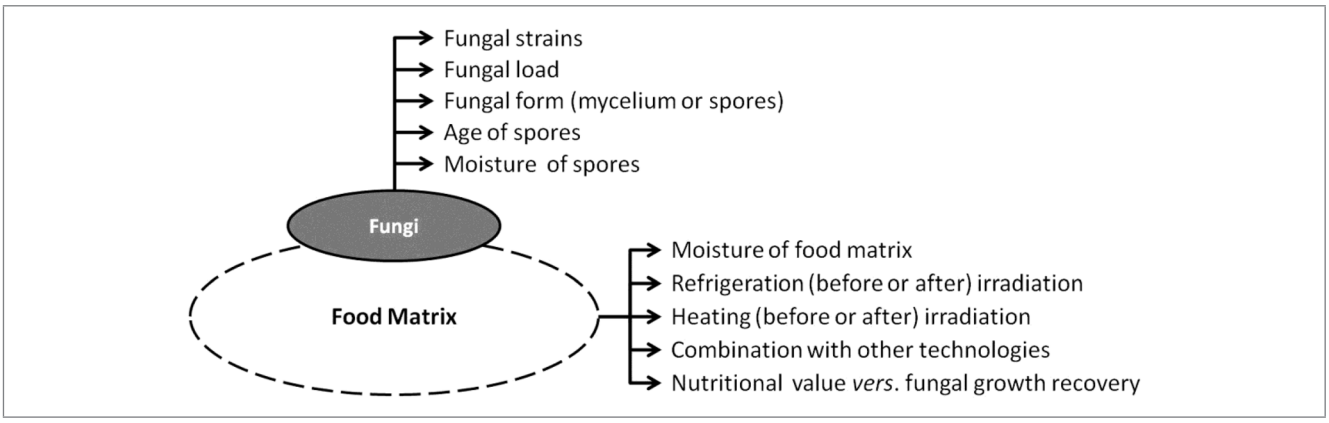

Figure 3–Factors that may influence the effectiveness of the spore irradiation process in food.

55%, depending on the mycotoxin) were observed in peppers with the highest moisture level (18%) and were irradiated at 30 kGy. Authors also observed that AFB_2 and AFG_2 were more radioresistant than AFB_1 and AFG_1. OTA was the most radiosensitive. In contrast, Hooshmand and Klopfenstein (1995) did not verify any reduction in AFB_1 in soybean, corn, and wheat irradiated with doses up to 20 kGy at 9%, 13%, or 17% moisture content, respectively. Nonetheless, these authors verified significant reductions in DON and ZEN concentrations at doses of 10 and 20 kGy, respectively, and in T-2 toxin with 7.5, 10, and 20 kGy doses. With an irradiation dose of 10 kGy, the maximum allowable for food products, eliminations of 16% for T-2 toxin in wheat, of 33% for DON in soybeans, and of 25% for ZEN in corn were observed, and with a 20-kGy dose, reductions were 20%, 41%, and 31%, respectively. The elimination of AFLs from yellow maize and from peanuts using gamma-radiation was also studied (Farag and others 2004). The experiments conducted showed that gamma-radiation at a dose of 20 kGy could eliminate 76% of AFB_1 in yellow maize and 85% of AFB_1 in peanuts. Reductions of 83% to 97% were also observed for the other AFLs at identical conditions. Prado and others (2003) also studied gamma-radiation effects on peanuts. Doses of 15 to 30 kGy were sufficient to eliminate AFB_1 by 55% to 74%. Nonetheless, these authors did not observe any increased effect with increasing irradiation doses. In maize, Aquino and others (2005) observed that an irradiation dose of 10 kGy could completely eliminate the presence of AFB_1 and of AFB_2 in samples. In contaminated feeds, Herzallah and others (2008) found that AFB_1 and total AFL contents decreased by 43% and 40%, respectively, with an irradiation dose of 25 kGy.

Most of the studies in the literature that reported on the impact of radiation on mycotoxins addressed AFs; however, studies with other mycotoxins can also be found, such as the effect of gamma-radiation on patulin in apple juice concentrate (Zegota and others 1988). With up to a 2.5-kGy dose, the elimination of patulin was partial and proportional to the irradiation doses. Beyond 2.5 kGy, patulin was completely eliminated. Similar results were obtained in an aqueous solution. At the tested conditions, irradiation did not change the titratable acidity, reducing sugars, carbonyl content, or amino acid composition of the juice. Yun and others (2008) corroborated this result in apple juice because these authors observed a reduction of 81% of patulin with a 3-kGy dose and almost total elimination with 5 kGy. These researchers also investigated the effect of irradiation on patulin in water and observed that a 1-kGy dose was sufficient to completely eliminate patulin. Nonetheless, these authors also observed that organic acids, such as malic, lactic, or ascorbic acid, and amino acids, such as serine, threonine, or histidine, conferred a protective action on the radiolytic degradation of patulin.

Pure OTA, which was dissolved in methyl alcohol, was also tested and stable, even at 75 kGy (Paster and others 1985). In contrast, OTA was sensitive to irradiation in water and in other aqueous solutions by Kostecki and others (1991), who reported that up to 50% of OTA was decomposed after gamma-irradiation. Similar results were obtained by Deberghes and others (1993), who reported that 50% of OTA in solution was also eliminated with doses of 2 and 3 kGy and that the elimination percentage increased to 80% when 4 and 5 kGy were used. Kumar and others (2012) irradiated OTA in powder form, in aqueous and methanolic solutions. In aqueous solution, 30%, 79%, and 93% of the OTA were eliminated with doses of 1, 2.5, and 5 kGy, respectively. Nonetheless, OTA was more resistant to irradiation when dried or when in methanolic solution. With 10 kGy, only 24% of the OTA was eliminated in methanol, and almost none disappeared in the powder form. The total elimination of OTA in feedstuffs was achieved with irradiation doses of 15 and 20 kGy in yellow corn and in soybeans, respectively, but not in cottonseed cake and feed concentrates, for which the elimination reached only a maximum of 47% (Refai and others 1996). In green coffee beans, at a 10-kGy dose, OTA degradation increased with the moisture content of samples (Kumar and others 2012). Reductions of 5%, 9%, 20%, 90%, and 100% in initial amounts of OTA were observed in coffee beans with moisture contents of 9%, 10%, 12%, 23%, and 58%, respectively.

The irradiation effect on DON and on 3-acetyl DON (3–ADON) was tested by O'Neill and others (1993) on maize in aqueous solution and in the dry state. These authors found that both mycotoxins were more sensitive to irradiation when in aqueous solution than on maize. In aqueous solution, both mycotoxins were completely destroyed by 50 kGy, and their breakdown began at 1 and at 5 kGy for DON and for 3–ADON, respectively. When irradiated on maize, breakdown only began after 20 kGy. In the dry condition, both mycotoxins were stable to irradiation at 50 kGy. Using electron bean irradiation, Stepanik and others (2007) also demonstrated a dose-dependent reduction of DON contents in wet distiller grains used as feed supplement. Reductions reached 47.5% to 75.5% at the highest doses (about 50 kGy). In contrast, the treatment of dry unprocessed wheat produced only a 17.6% reduction in the DON level at the highest dose, and the treatment was ineffective on dried distillers' grains. The effect of irradiation on *Fusarium* mycotoxins in wheat, flour, and bread

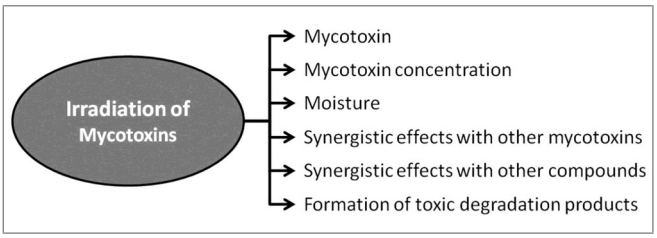

Figure 4–Factors that may influence the effectiveness of the mycotoxin irradiation process.

was also studied (Aziz and others 1997). DON, ZEN, and T-2 toxin concentrations were reduced with increasing doses of irradiation, but T-2 toxin was the most resistant to radiation. All mycotoxins were completely eliminated with an 8-kGy dose, and approximately 80% could be eliminated with a 6-kGy dose. Bread prepared from 6-kGy treated wheat flour was contaminated by DON, ZEN, and T-2 toxin with levels below 5 μg/kg of these mycotoxins (initial content was 272 μg/kg).

Fumonisin B_1 was also investigated. D'Ovidio and others (2007) studied the effect of irradiation on FB_1 in aqueous solutions and in corn. FB_1 in aqueous solutions was reduced by 99% using only 0.5 kGy; however, irradiation did not significantly reduce levels of this mycotoxin in whole and ground corn using irradiation doses up to 30 kGy. In contrast, Visconti and others (1996) reported that a 15-kGy dose caused a decrease in fumonisin contents of approximately 20% in maize flour. Better elimination of FB_1 was obtained by Aziz and others (2007) in wheat, maize, and barley grains, and the application on these grains of a radiation dose of 5 kGy inactivated FB_1 by 97%, 87%, and 100%, respectively. A dose of 7 kGy was sufficient for the complete destruction of FB_1 in wheat and maize.

In feedstuffs and feed samples, the influence of irradiation on *Penicillium* mycotoxins was studied by Aziz and Mattar (2007). Ten kGy eliminated citrinin contents up to 97.5% and eliminated OTA up to 78.5%. Patulin, cyclopiazonic acid, and rubratoxin B were not detected after irradiating commodities with a 5.0-kGy dose. Considering the reviewed studies, in Figure 4, we summarize the factors that must be considered to apply the irradiation process to mycotoxins.

Conclusions

Although there are several contrasting reports regarding the effect of gamma rays on fungi and mycotoxins in different foods, gamma-irradiation can generally be considered to significantly improve the mycotoxicological safety of food and feed. Indeed, gamma-irradiation has an inhibitory effect on mycotoxigenic fungi, inhibiting or delaying their development and, consequently, the production of mycotoxins, and under appropriate conditions, gamma-irradiation can directly destroy mycotoxins. Nonetheless, irradiation should only be used in combination with good manufacturing and storage practices to prevent the proliferation of toxigenic fungi and the associated production of mycotoxins. Also important is that irradiation should never be used in commodities already molded or contaminated with mycotoxins with the intent of remediating the problem.

The following is a brief summary of main key points that may be drawn from the reviewed literature:

- The radiolytic process is influenced by many factors, such as absorbed doses, initial mycotoxin concentration or fungal load, the position in the irradiated system, the amount of moisture, and/or the presence of other matrix components.
- Radiosensitivity of fungi also depends on strain characteristics, mold forms (mycelium or spores), the moisture content of spores or commodities, spore age, commodity characteristics, the existence of periods of refrigeration or of heating before or after treatments, and on the combinations of radiation with other technologies. Fungi with melanized mycelia and spores are also more radioresistant than other structures. Commodities with higher moisture content may favor fungal recovery after irradiation if inactivation is not complete.
- The fungal load may be substantially reduced with irradiation levels of 5 kGy and above; however, lower radiation doses can also be effective if products are previously treated with hot water.
- Irradiated fungal strains can occasionally produce more mycotoxins than original strains; however, appropriate storage after irradiation can minimize the development of remaining fungal propagules.
- Dried mycotoxins are extremely radioresistant, whereas in solution, mycotoxins are sensitive to irradiation. The oxidative radicals that originate from water radiolysis are responsible for their degradation.
- Combining gamma-irradiation with other treatments can improve the breakdown of mycotoxins (for example, using hydrogen peroxide, ammonium bicarbonate, or higher moisture conditions).
- Generally, more than 10-kGy doses are required to eliminate a significant amount of mycotoxins in food matrixes. Patulin is an exception because patulin can be completely destroyed in apple juice by radiation doses between 2.5 and 5 kGy.
- The loss of toxicity after irradiation was only demonstrated for AFB_1.

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