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Effects of Ionizing Radiation on Microbiological Contaminants of Foods

Giorgiana Mihaela BELBE, Maria TOFANĂ

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture,
3-5 Mănăştur street, Cluj-Napoca, România
e-mail: giorgiana.belbe@gmail.com

Abstract. Ionizing radiation can be used in food industry in order to control the number of microbiological contaminants, among others. The sources of irradiation allowed to be used in this respect are: high-voltage electron beams up to 10 MeV, X – rays up to 5 MeV and gamma rays produced from the radioisotopes ^{60}Co and ^{137}Cs.

One of the most important properties of irradiation is inactivation of microorganisms, especially pathogens. Gram-negative pathogen bacteria are very sensitive to radiation. As expected bacterial spores are more resistant to ionizing radiation than vegetative cells are. Irradiation also reduces the number of mould populations. Yeasts on the other hand are more radio-resistant than bacteria and moulds and can become the dominant flora of irradiated foods. In what viruses are concerned, ionizing radiation must be accompanied by other treatments in order to be an efficient inactivation method.

In conclusion, ionizing radiation can be used, at acceptable doses, as a control method in preserving the innocuity of foods.

Keywords: ionizing radiation, gamma rays, X–rays, accelerated electrons, irradiated foods, biological contaminants, preservation methods.

INTRODUCTION

Irradiation of food is a physical process that uses ionizing radiations from radioactive isotopes of cobalt or cesium or from accelerators that produce controlled amounts of gamma rays, X rays or electron beams to assure the innocuity of foods and to prolong the shelf life.

As the name points out, the main effect of the ionizing radiation on the irradiated material is ionization. Radiations can strip electrons away from atoms or molecules that are left positively charged (ionized) (Moise, I. V., 2009).

$$\gamma + {}_{A}^{Z}X \xrightarrow{\text{Photoelectric effect}} {}_{A}^{Z-1}X + e^{-}$$

$$\gamma + {}_{A}^{Z}X \xrightarrow[\text{Compton effect}]{} {}_{A}^{Z-1}X + e^{-} + \gamma$$

If the atom is part of a molecular structure, and the ejected electron is part of a chemical moiety charged species will appear in the irradiated material. The charged species rapidly split into free radicals that have a free or unpaired electron. Free radicals react extremely rapidly with each other and nearby molecules as they seek to become more stable by gaining or losing an electron. It is these free radical reactions that trigger the chemical effects leading to the ultimate changes in the food.

$$H_2O \xrightarrow{\gamma} OH^{-} + H^{+}$$

Ionizing radiation (gamma rays, X-rays) has a very short wavelength and high energy, high enough to change atoms by eliminating an electron from them and forming an ion, but not high enough to split atoms and cause exposed sources to become radioactive (Molins, R. A., 2001).

The absorbed dose is proportional to the ionizing energy absorbed per unit of irradiated material. The effects of the treatment are related to this quantity, which is the most important specification of any irradiation process. (McLaughlin, W. L., 1989).

The definition of absorbed dose can be expressed by the following equation (Sommers, C. H. and X. Fan, 2006):

$$Da = \frac{F_P PT}{M} \qquad \textbf{(1)}$$

D_a – the average dose, (kGy)
F_p – a dimensionless factor that accounts for the fraction of emitted power absorbed by the material, $F_p \in [0.25;0.50]$
P – the emitted power of the radiation source, (kW)
T – the treatment time, (s)
M – the mass of the material, (kg)

Absorbed dose requirements for various industrial irradiation processes cover a wide range from 0.1 kGy to 1000 kGy. Most of these processes need less than 100 kGy, while many need less than 10 kGy and some need less than 1 kGy.

The absorption of energy during irradiation can provide foods with desirable new benefits:
• inhibit sprouting
• destroy insects and parasites
• delay the ripening and spoilage
• extend the shelf-life of perishable
• eliminate pathogen microorganisms in food
• sterilize at doses above 10 kGy

Radiations used in food industry

The one overriding requirement for an energy source to be employed in food irradiation is that the energy levels must be below those that could possibly cause the food to become radioactive. After that requirement is met, sources are considered on the basis of their practical and economic feasibility (Jones, J. M., 1992).

There are two principal forms of ionizing radiation: accelerated subatomic particles and high-energy electromagnetic (EM) radiation.

a. Accelerated Particles

Particles travelling at speeds approaching the speed of light can cause ionization. The only particle used for food irradiation is the electron (Roberts, P. B., 2003). High-voltage electron beams (E-beams), accelerated electrons, generated from linear accelerators are an alternative to radioisotope generators.

In order to prevent activation (production of radioactive isotopes) a maximum limit of 10 MeV was established for the energy of E-beams (Moise, I. V., 2009).

b. Electromagnetic Radiation

There is a range of EM radiations each characterized by different wavelengths or energy and with different uses. Only X- rays and gamma-rays have sufficient energy to cause ionization (Roberts, P. B., 2003).

X–rays are EM radiations whose energetic spectrum overlaps that of gamma radiations. Of interest in the ionizing radiation treatment are bremstralung X– rays which appear when accelerated electrons are stopped in the electric field of the irradiated target. For the same reasons as in the case of E-beams, the X–rays energy has to be restrained to 5 MeV (Moise, I. V., 2009).

Gamma-rays are emitted by many atomic nuclei undergoing radioactive decay. The gamma rays sources that are permitted for use in food irradiation are produced from the radioisotopes ^{60}Co (1.17 and 1.33 MeV) and ^{137}Cs (0.662 MeV).

The choice of a radiation source for a particular application depends on practical aspects such as thickness and density of the material, dose uniformity ratio, minimum dose, processing rate and economics.

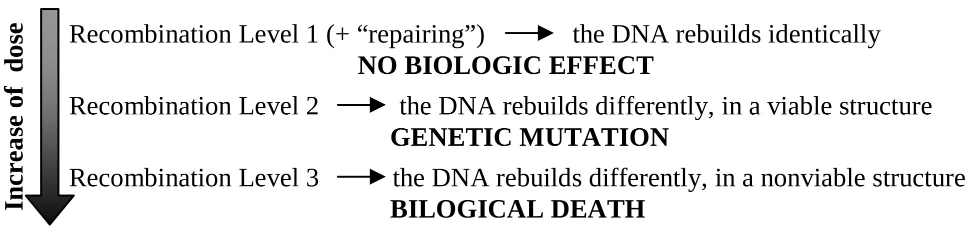
Effects on microbiological contaminants

The biocide effect of ionizing radiations was observed soon after their discovery. Dickson (2001) explained that the first noticeable effect of ionizing radiation is DNA lesion or recombination. This damage prevents multiplication, randomly inhibits cell functions and, depending on the number of affected cells and the complexity of the organism, can cause death. 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 the DNA ‘target’ in the microorganism is one of the most important factors.

Apart from affecting the DNA, ionizing radiation can also act on an adjacent molecule which subsequently reacts with the genetic material. Irradiation may affect other cell components, such as the membrane, the enzymes and the cytoplasm. This damage may not be directly lethal, but can damage the cell sub lethally, so that it can impede the survival of the damaged cell (Pollard, E. C., 1966).

Obviously, the biocide effect depends on the absorbed dose. At low doses chemical reactions can disrupt bio-chemical and hormonal processes in fresh produce. As the dose increases, cell division is prevented as chemical disruption of DNA becomes severe (Lacroix, Monique, 2005). In this way microorganisms are. At the highest doses used, all the microorganisms are killed and the food is sterilized.

Fig. 1. represents the dominant biological effect of radiation in realation to the absorbed dose.



(Moise, I. V., 2009)

Fig. 1. Biological effect of radiation in realation to the absorbed dose

According to Frazier and Westhoff (1988), the bactericidal efficacy of a given dose of irradiation depends on the following:

1. the kind and species of the organism, this factor is presented in Tab. 1, Tab. 2 and Tab. 3.

2. the numbers of organisms (or spores) originally present, the more organisms there are, the less effective a given dose will be

3. the composition of the food, some constituents (e.g., proteins, catalase, nitrites, sulfites, sulfhydryl compounds) may be protective, compounds that combine with the SH groups would be sensitizing

4. the presence or absence of oxygen, the effect of free oxygen varies with the organism, ranging from no effect to sensitization of the organism

5. the physical state of the food during irradiation, both moisture content and temperature affect different organisms in different ways, see Tab. 4

6. the condition of the organisms: age, temperature of growth and sporulation, and state (vegetative or spore) may affect the sensitivity of the organisms, see Tab 1.

Effects on bacteria

One of the most important properties of irradiation is inactivation of microorganisms, especially pathogens.

Those microorganisms which are efficient in repairing their DNA show the greatest probability of survival, and are therefore the most resistant to irradiation. In addition to this survival mechanisms there are microorganisms which are resistant to radiation owing to the fact that they possess a mechanism for damaged genetic material elimination, which aids their survival. One example of a bacterium resistant to radiation is *Deinococcus radiodurans*, which can be found in foods which have been irradiated with doses up to 40 kGy (Dickson, 2001).

It has been observed that the number of microorganisms present in an irradiated food decreases with the applied dose (Ibarz, A., 2008):

$$N = N_0 \exp(-kD) \qquad \textbf{(2)}$$

N – the number of microorganisms which survive
N_0 – the number of microorganisms initially present in the food
k – the kinetic constant of microorganism destruction by irradiation
D – the dose applied

Fig. 2 presents the typical bacterial survival curve following irradiation and Fig. 3 shows the bacterial radio-inactivation curves for some representative bacteria.

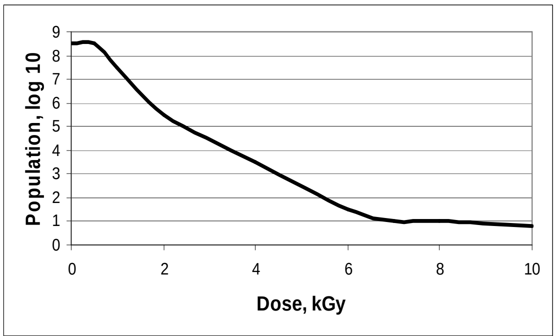


Fig. 2. Typical bacterial survival curve following irradiation (Molins, R. A., 2001)

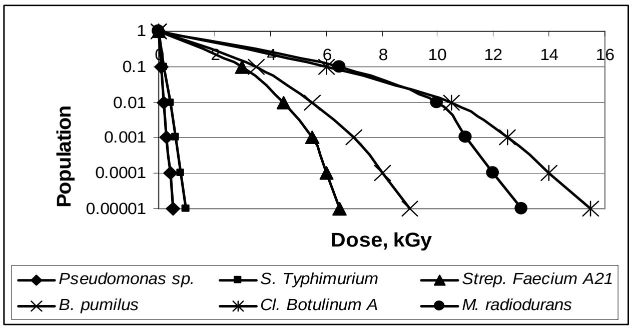


Fig. 3. Bacterial radio-inactivation curves for some representative bacteria (IFIN HH.)

As shown in Tab. 1, Gram-negative saprophytes bacteria involved in food spoilage are radio-sensitive. In general Gram-positive spoilage bacteria, lactobacilli and lactococci, are more radio-resistant than Gram-negative spoilage bacteria. Gram-negative pathogen bacteria, *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Campylobacter spp.* are very sensitive to radiation. Of all, *Salmonella* is the most radio-resistant. So, a treatment designed to eliminate *Salmonella* will ultimately destroy all Gram-negative pathogens. Bacterial spores are more resistant to ionizing radiation than are vegetative cells.

While research was conducted on ionizing radiations, the problem of increase in pathogenesis and radio-resistance of irradiated microorganisms arose. It was not found any scientific proof that ionizing radiations treatment increases microorganisms' pathogenesis, virulence and radio-resistance. Moreover, the International Committee on Food Microbiology and Hygiene, (ICFMH) after a meeting in Copenhaga in 1982, concluded that food irradiation is an important pathogens control method and that it does not present any risks for consumers (Barbosa-Canovas, G. V., 1998).

Tab. 1

Approximate killing doses of ionizing radiations in kiloGrays (kGy)

Organism	Approximate lethal dose (kGy)	Organism	Approximate lethal dose (kGy)
Bacterial pathogens in vegetative state			
Gram-negative:			
Salmonella spp.	3.7 - 4.8	*Escherichia coli O157:H7*	0.24-0.47
Camlylobacter jejuni	0.08-0.32	*Yersinia enterocolitica*	0.04-0.39
Gram-positive			
Mycobacterium tuberculosis	1.4	*Clostridium perfringes*	0.29-0.85
Corynebacterium diphtheriae	4.2	*Listeria monocytogenes*	0.25-0.77
Bacillus cereus	0.02-0.58	*Staphylococcus aureus*	0.26-0.45
Bacterial saprophytes in vegetative state			
Gram-negative:			
Escherichia coli	1.0 - 2.3	*Pseudomonas putida*	0.08-0.11
Pseudomonas aeruginosa	1.6 - 2.3	*Enterobacter aerogenes*	1.4 - 1.8
Pseudomonas fluorescens	1.2 - 2.3	*Moraxella phenylpyruvica*	0.63-0.88
Gram-positive			
Lactobacillus spp.	0.23 - 0.38	*Leuconostoc dextranicum*	0.9
Streptococcus faecalis	1.7 - 8.8	*Sarcina lutea*	3.7
Bacterial spores:			
Bacillus subtillus	12 - 18	*Clostridium botulinum* (E)	15 - 18
Bacillus coagulans	10	*Clostridium perfringens*	3.1
Clostridium botulinum (A)	19 - 37	*Putrefactive anaerobe* 3679	23 - 50

(Barbosa-Canovas, G. V., *et al.*, 1998, Radomyski, T. *et al.*, 1994; Monk, M. L., *et al,* 1995)

Effects on moulds

Irradiation reduces the number of mould population, but their effects on mycotoxin production of surviving moulds are not well understood (Monk, M. L., 1995). Irradiation can lead to a growth of mycotoxin production as the inactivation of concurrent micro flora could allow the moulds to increase it. But a reduction of could also be expected.

Tab. 2

Radio-resistance of some mould species, irradiated at room temperature

Mould Species	Necessary dose to prevent mould growth (kGy)	Mould Species	Necessary dose to prevent mould growth (kGy)
Aspergillus flavus	1,6	*Botrytis cinerea*	5,0
A. niger	2,5	*Cladosporium* spp.	6,0
A. parasiticus	1,6	*Penicillium viridicatum*	1,4
Alternaria spp.	6,0		

(WHO, 1999)

As summarized in Tab. 2, *Aspergillus* and *Penicillium* genii are among the most radio-sensitive moulds. In what dried foods and ingredients contaminated with *Alternaria alternate, Cladosporium cladosporoides* or *Culvularia* spp., are concerned, irradiation with doses up to 10 kGy will not guarantee mould destruction. But, following the GAP and GMP before irradiation, mould contamination will be kept within proper limits.

In conclusion, foods with high moisture content irradiated at high doses can be mould sterilized using ionizing radiation.

Effects on yeasts

Although yeasts are not usual agents for food born diseases, their presence and development in some foods can create favorable multiplication conditions for other spoilage or pathogenic microorganisms. Secondly, there can be pathogenic yeasts vehiculated by foods such as *Candida albicans* and *Cryptococcus neoformans*.

Most yeasts are radio-sensitive, with D_{10} values of 0.1 to 0.5, that means that a dose of 5 kGy will reduce the existing yeast population by at least 10D (Wilkinson, V. M. and G. V. Gould, 1998). There are radio-tolerant strains, tough. Stehlik and Kaindl (1966) studied a radio-resistant strain of *Saccharomyces cerevisiae* var. *ellipsoideus* which had a D_{10} value of 3 kGy when irradiated at 20°C. The rate of inactivation decreased significantly with the increase of temperature, so that at 45°C, the D_{10} value was only 0,5 kGy.

Tab.3

Radio-resistance of some yeast at gamma ionizing radiation

Yeast	Necessary dose prevent yeast growth (kGy)	Yeast	Necessary dose prevent yeast growth (kGy)
Candida crusei	5.5	*Saccharomyces carlsbergiensis*	15
C. tropicalis	10	*S. cerevisiae*	18
Cryptococcus albidus	10	*S. rosei*	15
Debaryomyces klöckeri	7.5	*Sporobolomyces pararoseus*	5
Pullularia pullulans	20	*Torulopsis stellata*	10
Rhodotorula glutinis	10		

(WHO, 1999)

Analyzing the data in Tab. 3, it can be said that because of their radio-tolerance some yeasts can become the dominant flora of irradiated foods if they are present in a high enough number.

Effects on Viruses

Viruses are the smallest pathogens with nucleic acids. They are, in general, more radio-resistant than other microorganisms with D_{10} values larger than 10 kGy (Young, A. L., 2003). Radio-resistance of viruses can grow up to 10 times that of bacteria depending on factors such as: concentration of organic material in the medium, irradiation temperature and moisture content.

The necessary doses for the inactivation of viruses increase with the decrease of their dimension, reaching levels of 20-100 kGy. Mallet *et al.* (1991) suggested a dose of 3.1 kGy to eliminate 90% of A hepatitis virus, poliovirus and rotavirus in shellfish and Sullivan *et al.* (1973) proposed a dose of 6.8 kGy to inactivate type B2 coxsackievirus in ground beef. The radio-resistance of coxsackievirus type B2 to gamma ionizing radiation is presented in Tab. 4.

Tab. 4

Radio-resistance of coxsackievirus type B2 to gamma ionizing radiation

Irradiation environment	Irradiation temperature (°C)	D_{10} values (kGy)	Irradiation environment	Irradiation temperature (°C)	D_{10} values (kGy)
water	0,5	1,4	Cooked beef	-30	6,8
	-90	5,3		-60	7,8
Cooked beef	16	7,0		-90	8,1
	0,5	7,6			

(Sullivan *et al.,* 1973)

It is important to keep in mind that, in the majority of cases, a 90% inactivation of virus population is not enough to eliminate the possibility of infection. But, a combination of ionizing radiation with other treatments such as a mild thermal treatment or a microwave treatment, additive addition or irradiation in ozone atmosphere can improve the efficiency of virus' inactivation.

CONCLUSIONS

Irradiation of food must not be confused with the contamination of food by radioactive materials, which themselves emit radiations that may harm the consumer. Food irradiation cannot make food radioactive since the radiation used, though of high energy, is not powerful enough to induce the necessary changes in atomic nuclei.

The radiation sources accepted in food industry are: gamma rays from radio-nuclides ^{60}Co or ^{137}Cs, X-rays with nominal energy level below 5 MeV and accelerated electrons with nominal energy level below 10 MeV.

Ionizing radiation has a biocide effect because it affects either the DNA or major cell components the contamination flora. Consequently, it can be used in order to control the microorganism number in foods. So, it can inactivate microorganisms, especially pathogens. It was observed that Gram-negative pathogen bacteria are very sensitive to radiation, *Salmonella* being the most radio-resistant. As expected bacterial spores are more resistant to ionizing radiation than vegetative cells are.

Irradiation also reduces the number of mould populations.

Yeasts on the other hand are more radio-resistant than bacteria and moulds and can become the dominant flora of irradiated foods.

In what viruses are concerned, ionizing radiation must be accompanied by other treatments, such as thermal or microwave treatment or additive addition in order to be an efficient inactivation method.

While irradiation provides many benefits, it cannot replace proper food handling as the single most critical food safety measure. Irradiation does not prevent contamination but it controls it.

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